UNIVERSITY OF PISA

Research Doctorate School in Biological and Molecular Sciences

(2010-2012)

"Identification and characterization of genes involved in the Malignant Pleural Mesothelioma"

SUPERVISOR

Prof. Stefano Landi

PhD STUDENT

Ombretta Melaiu

Index

Abstract

Malignant pleural mesothelioma (MPM) is an asbestos-related cancer of the pleural cavities. Since the molecular mechanisms of MPM are poorly understood, our main goal was to identify relevant genes involved in this neoplasm. Firstly, we performed an extensive literature review focused on the MPM transcriptome and a data mining (using Coremine, SNPs3D, and GeneProspector). The results from review of transcriptome studies and from data mining were intersected. Then, we undertook a validation study to verify whether 77 genes could be confirmed in their de-regulation on an independent series of specimens (20 MPM and 20 healthy pleura tissues). The genes resulted deregulated in our MPM and healthy pleural tissues, have been further validated on four MPM cell lines. The high expression levels of Mesothelin (*MSLN),* Calretinin (*CALB2*), and Platelet-derived growth factor receptor beta (*PDGFRB*) captured our interest. *PDGFRB* is a target for the tyrosine-kinase inhibitor imatinib. Personal communication (reported by Dr. L. Mutti, Hospital of Vercelli, Italy) highlighted that imatinib was assayed for compassionate use in advanced MPM patients in combination with gemcitabine. Preliminary observations reported that patients showed a good response with the stabilization or partial shrinkage of the tumor mass. However, some patients either did not respond or, after an initial response, relapsed. We hypothesized that mutations within *PDGFRB*, occurring during the carcinogenesis or during the therapy, could explain these observations but no mutations were found within *PDGFRB* in 100 surgically resected MPMs. Imatinib resistant Mero-14 cell lines did not show mutations within *PDGFRB*, making difficult to understand the resistance to imatinib. In order to prove that *MSLN* and *CALB2* play a role in maintaining the malignant phenotype, rather to be simply epiphenomenons, we attempted an approach using silencing-RNA. After having switched off these targets, we analyzed the behavior of MPM cell lines for their apoptotic ability, invasion capacity, cell cycle, and in culture growth parameters. As regard the *CALB2* depletion, from our findings we can hypothesize that this gene does not seem involved in triggering the disease. For *MSLN*, its depletion causes the arrest of some of the most important characteristics malignant phenotypes. Indeed, the specific gene silencing for *MSLN* decreased the viability, and the invasiveness of MPM cells. Moreover we showed that *MSLN* depletion sensitized Mero-14 cell lines to cisplatin, and that under this treatment, they displayed an apoptotic type of cell death, and a substantial arrest of the proliferation rate.

Finally, the most important result of this work provides evidence for a possible targeting of *MSLN*, alone or in combination with chemotherapy, for the treatment of MPM, highlighting the importance of this target gene for novel therapies.

1. Introduction

1.1 Background

Malignant pleural mesothelioma (MPM) is a rare cancer of the pleural cavity triggered by asbestos exposure. Besides the clear established role of the asbestos, other risk factors were suggested, such as the exposure to ionizing radiations (Zucali and Giaccone 2006; Carbone and Bedrossian 2006), the infection to SV40 (Rivera et al., 2008), as well as an enhanced individual genetic susceptibility (Carbone et al., 2012; Roushdy-Hammady et al., 2001; Landi et al., 2007). At the present time, the overall outcome of this disease remains not encouraging: indeed for the majority of patients, MPM is diagnosed at late stages when the tumor remains unresectable, the prognosis is poor, and an efficient management strategy for this cancer is still absent.

It is well known that cancer occurs after a progressive accumulation of mutations in several genes, leading to an altered expression of oncogenes and tumor-suppressor genes that are directly involved in tumorigenesis. In recent decades, in order to define the molecular aspects of MPM, various studies have been conducted. Genome-wide array-based approaches have allowed progress in MPM research by identifying changes at the genetic (Ivanov et al., 2009) and epigenetic (Goto et al., 2009) levels. Transcriptomic studies, classically performed by microarrays, have led to the identification of different expression patterns relevant to the biology of several types of cancers such as ovarian carcinoma (Chon and Lancaster, 2011), acute myeloid leukemia (Godley et al., 2011), diffuse large B-cell lymphoma (Alizadeh et al., 2000), and hepatocellular carcinoma (Woo et al., 2011). Altered gene expression has been already described for asbestos-induced mice tumors (Zhao et al., 2000; Sandhu et al., 2000) and for cell lines reproducing the MPM progression (Rihn et al., 2000; Kettunen et al., 2001). Additionally, gene expression profiling has been suggested to allow the differential diagnosis between MPM and lung cancer and to monitor the response to treatments (Gordon et al., 2003). The identification of specific gene expression changes in cancer mesothelial cells may lead to a better understanding of the pathogenesis of MPM and generate new candidate biomarkers with potential clinical value. At the moment, indeed, high sensitive and specific biomarkers for MPM, useful for clinical practice, are not known except for a few proteins like SMRP (serum soluble mesothelin-related peptide) and the osteopontin, which are still under investigation (Robinson et al., 2003; Cristaudo et al., 2007; Grigoriu et al., 2007).

1.2 The normal mesothelium

The mesothelium is a membrane, consisting of a monolayer of specialized pavement-like cells that line the body's serous cavities and internal organs. It takes the name of pleura, if it surrounds the lung, peritoneum for the abdominal cavity, and pericardium for the heart sac. Mesothelial tissue also surrounds the male, and female internal reproductive organs (the tunica vaginalis testis, and the tunica serosa uteri, respectively). The visceral mesothelium is the mesothelium that covers the internal organs, while the layer that surrounds the body walls is the parietal mesothelium (Mutsaers, 2002). Mesothelial cells are derived from the embryonic mesoderm cell layer, that lines the coelom (body cavity) in the embryo (Tiedemann, 1976). Numerous are their functions. The mesothelium forms a barrier against pathogen agents, and injury due to the inflammation, keeping the integrity of the serosal space, it surrounds (Mutsaers, 2004). The second important function of the mesothelium is the transport of fluid and cells across the serosal cavities.

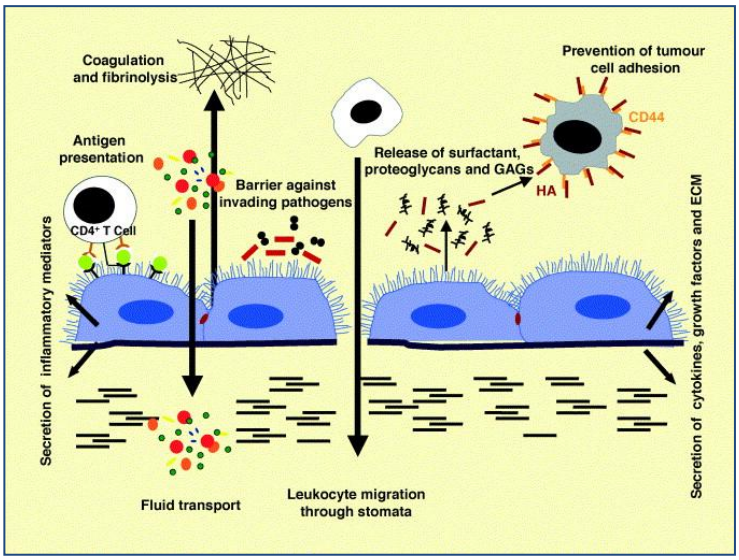

Figure 1: Functions of mesothelial cells. (Mutsaers, 2004)

1.2.1 The pleura

The pleura is a thin serous membrane which turns back on itself to form a double layer. The outer pleura (parietal pleura) is wrapped around the chest wall. The inner pleura (visceral pleura) covers the lungs and adjacent structures, ie. blood vessels, bronchi, and nerves. The pleurae are continuous with each other at the hilum. The parietal pleura is divided into three parts: rib, which adheres to the coast; mediastinal, which lies medial to the heart; diaphragm, which is located along the diaphragm. The visceral pleura starts from the hilum into the lung, along the bronchi and blood vessels, and

allows the distinction in the pulmonary lobules. The visceral and parietal pleura delimit a space, called "pleural cavity", containing the pleural fluid which, during the respiratory activity, allows the two pleurae to slide over each other. Between the two layers there is a negative pressure (vacuum Donders), required to keep the lung stretched, within the pleural sac, and to allow the modification of volume during breathing movements. Pleural fluid is a serous fluid produced by the normal pleurae in order to provide a slippery, non-adhesive, and protective surface to facilitate intra-coelomic movement (Peek et al., 2000). The parietal layer secretes 2400 ml of fluid daily, which is reabsorbed by the visceral layer.

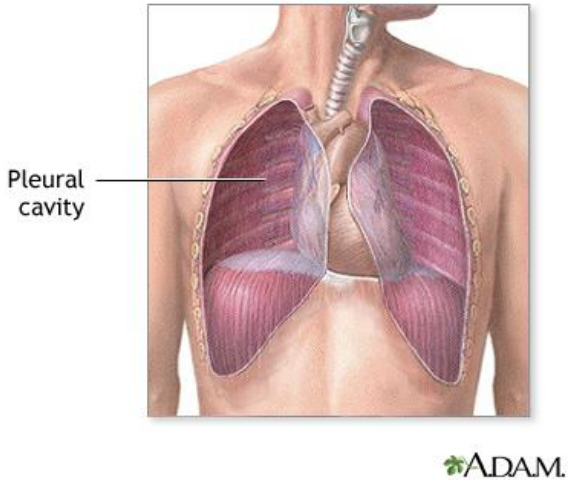

Figure 2: Pleural cavity

1.3 MPM: clinical features

The neoplastic cells are initially localized in the basal pleural segments, then the tumor spreads involving the pleural spaces together with a large pleural effusion, as well as with the direct invasion of the thoracic structures. The affected lung is permeated and surrounded by a thick layer of gelatinous neoplastic tissue. Three main histological subtypes are known: the majority of MPMs is of the epithelioid subtype (about 50%), 10% are sarcomatoid and the rest are biphasic/mixed (Travis et al., 2004.). These groups show significant differences in their histological and clinical features:

- Epithelioid: characterized by cuboidal, cylindrical or flattened cells, conferring to the structure a shape tubular, papillary, or tubulo-papillary. The epithelial cells have an acidophilus cytoplasm, and vesicular nuclei with prominent nucleoli.
- Sarcomatoid: it shows interwoven bundles of spindle cells (fibroblast-like) in a stroma collagen.

- Mixed (biphasic): it is the classical histological picture in which the two components, epithelial and sarcomatoid, coexist.

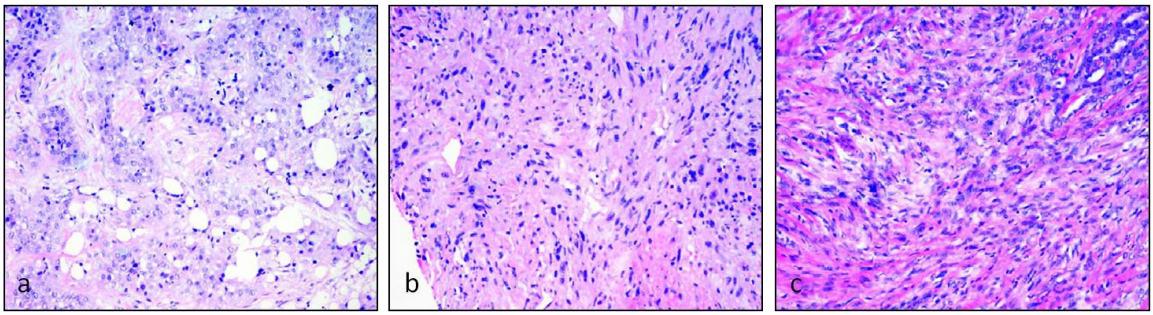

Figure 3: Haematoxylin-eosin staining to identify the histology of MPM a) Epithelioid MPM b) Sarcomatoid MPM c) Biphasic MPM

According to the TNM staging system, the evolution of the MPM goes through four stages (Moore et al., 2008):

1. Stage I (localized MPM): the cancer interested the right or left pleura and may also spread to the lung, pericardium, or diaphragm, on the same side. Lymph nodes are not involved.
2. Stage II: the cancer spreads from the pleura, on one side, to the lymph nodes next to the lung, on the same side. It may also spread to the lung, pericardium, or diaphragm, on the same side.
3. Stage III: the cancer invaded the chest wall, muscle, ribs, heart, esophagus, or other organs in the chest, on the same side, with or without lymph node involvement, on the same side as the primary tumor.
4. Stage IV: the cancer spreads to the lymph nodes in the chest on the side opposite to the primary tumor, or extends to the pleura or lung on the opposite side, or directly to the organs of the abdominal cavity or neck. At this stage, there are distant metastases.

1.4 Epidemiology of the MPM

Since MPM arises most frequently in workers exposed to asbestos fibres, this neoplasm is considered an occupational disease (Mazzoni et al., 2012). Indeed, it is thought that about the 80% of cases is caused by asbestos exposure (McDonald and McDonald 1996).

The first case of MPM has been described in 1947 (Yang et al., 2008). The relationship between MPM and asbestos was first documented in a study conducted in South Africa, on a group of miners of asbestos in 1960 (Wagner et al., 1960). From then, the scientific community has

continued to demonstrate the relationship between exposure to asbestos and development of MPM (Selikoff et al., 1968; Spirtas et al., 1994; Hansen et al., 1998; Kurumatani and Kumagai, 2008), and the developed countries started to collect epidemiological data on asbestos and MPM to confirm the relationship between the incidence of the disease, and the asbestos exposure.

The latency period for MPM after initial exposure to asbestos is typically longer than 30 years, and the median survival time after diagnosis is 9–12 months (Robinson et al., 2005).

For this reason, although the use of asbestos has been banned in the Western world since the 80s, the incidence of MPM is expected to increase in countries such as Italy (Peto et al., 1999), Great Britain (Tan et al., 2010), and Australia (Kao et al., 2011). On the contrary, the United States has already reached the peak of cases of MPM because asbestos was banned earlier than elsewhere (Price and Ware 2004), although it is expected a further increase in incidence in the aftermath of the World Trade Center (Ismail-Khan et al., 2006), which was partly built with asbestos (insulation, partitions).

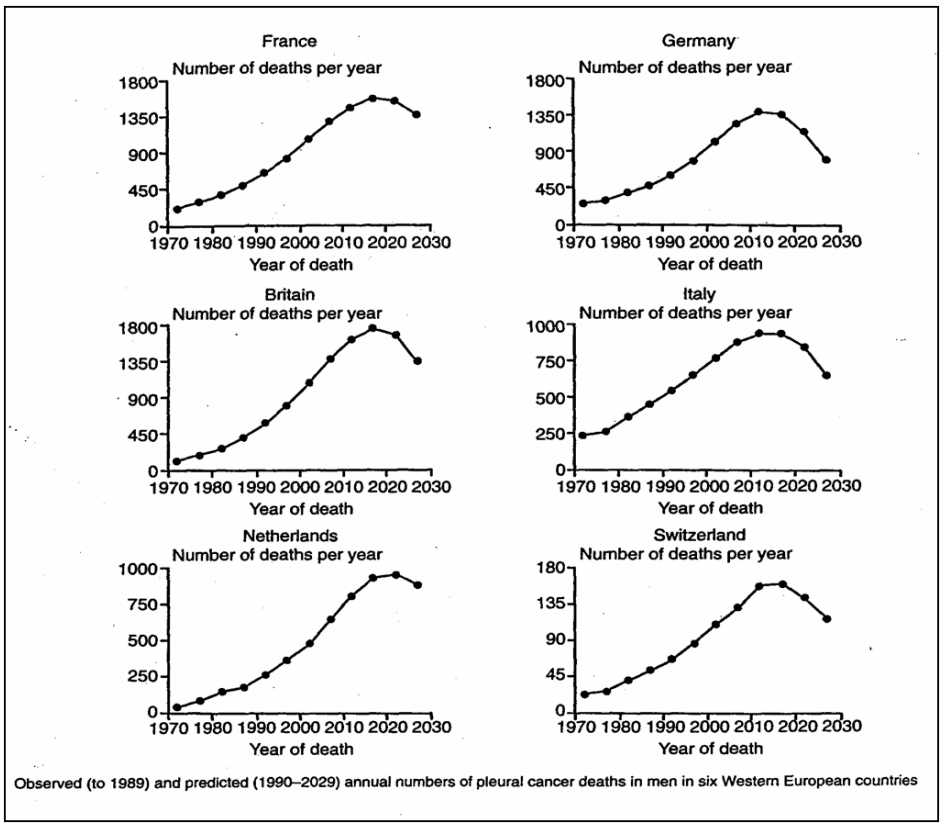

Figure 4: Estimations of MPM death in Western Europe countrie

Delgermaa et al., have summarized data on MPM death reported by the World Health Organization database since 1994 to 2008 (Delgermaa et al., 2011). However, it should be noticed that, because

the MPM is a rare disease, few international data are available. A brief extract of the recorded observations are reported below.

In total, 92.253 MPM deaths were reported by 83 countries. Crude and age-adjusted mortality rates were 6.2 and 4.9 per million, respectively. The mean age at death was 70 years, and it has been reported that the incidence is much higher in men than women. Regarding the geographical distribution of deaths, the United States of America reported the highest number, while over 50% of all deaths occurred in Europe. In contrast, less than 12% occurred in middle- and low-income countries.

Although the asbestos was banned or restricted in most of the industrialized world, its use is increasing in some parts of Asia, South America, and the former Soviet Union. The asbestos exports from Canada, Russia, Brazil to countries such as India, Indonesia, and Philippines, is creating an epidemic that may take decades to peak. Moreover, no data were available for China, India, Kazakhstan, the Russian Federation or Thailand, which have produced, or consumed asbestos, at substantial levels for many years.

Regarding the Italian background, in 1992 (Law No. 257), each mining, trade, import, export and production of asbestos, or products containing asbestos have been banned from the entire Italian territory. Epidemiological surveillance of MPM cases is entrusted to the National Register of Mesothelioma (ReNaM), established at the National Institute for Occupational Safety and Prevention (ISPESL). The third report of the ReNaM, published in 2010, describes the entire series of the reported MPMs, until 2004. 9,166 cases of malignant MPM have been counted. The average age at diagnosis is 68.3 years. The standardized rate for MPM is equal to 3.42 (per 100,000 individuals) in men, and 1.09 in women. In all the cases detected by the registry, 69.8% have occupational exposure, 4.5% family environment, 19.5% unlikely or unknown exposure. The number of cases varies considerably from region to region: Piedmont has the highest number of cases, followed by Liguria and Lombardy. Few cases have been reported in Calabria, Sardinia, and Abruzzo.

1.5 Aetiology of MPM

1.5.1 Asbestos

According to the World Health Organization (WHO), “asbestos is one of the most important occupational carcinogens causing about half of the deaths from occupational cancer”.

The term asbestos is a commercial designation which includes a group of mineral silicates fibres of the serpentine and amphibole series (Park et al., 2012). The basic forms of asbestos are reported in

the Table 1. The chrysotile is also known as white asbestos, the crocidolite as blue asbestos, and the amosite as brown asbestos (Straif et al., 2009).

ASBESTOS	
Serpentine	Amphibole
Chrysotile 3MgO.2SiO2.2H2O Orthochrysotile Mg3Si2O5(OH)4 Parachrysotile (Mg,Fe)3Si2O5(OH)4 Clinochrysotile Mg3Si2O5(OH)4	Amosite (FeMg)6SiO22(OH)2
Lizardite Mg3Si2O5(OH)4	Tremolite 2CaO.5MgO.8SiO2H2O
Antigorite (Mg,Fe)3Si2O5(OH)4	Anthrophyllite (FeMg)7SiO22(OH)2
	Actinolite (CaMgFe)6Si8O22(OH)2
	Crocidolite Nao.Fe2O3.3FeO.8Sio2.H2O

Table 1: The basic forms of asbestos

Widely used in the past, the asbestos during the 20th century was known as the "magic mineral". Indeed, the physical properties of asbestos - durability, flexibility, high tensile strength and resistance to heat, chemicals and electricity, made it suited for a number of commercial applications. It has been massively used to produce the Ethernit, a mixture of asbestos with cement for the insulation of buildings, roofs, ships, trains, and as a building material (tiles, floors, pipes, paint, chimneys), for the car (paint, mechanical parts), for the manufacture of ropes, plastic and cardboard. Furthermore, the dust of asbestos has been widely used as an adjuvant in the filtration of wine (Virta, 2003). The demand for asbestos peaked around 1977, with a production of almost 5 million metric tons per year. In the period from 2003 to 2007, the global production of asbestos, although it was reduced compared to the first, was fairly stable at 2.1 million metric tons per year. The majority of this volume was consumed by industrializing countries. Currently, 90% of asbestos is used to manufacture asbestos-cement, and it remains a popular raw material, for construction, in the developing world (Park et al., 2012).

All the mineral silicate fibres can theoretically cause the MPM, but, based on their mineralogical series and carcinogenicity, it is possible identify 2 main classes:

1. The amphibolic asbestos is the most carcinogenic. Their fibres are highly bio-persistent, they can stay indefinitely in the tissues, and have a high iron content. Crocidolite, and Amosite are the main types of asbestos used commercially, characterized by rigid and straight fibres, with higher physical

and chemical stability. Tremolite, Actinolite, and Erionite are not used commercially, and are naturally found in different zones (Afghanistan, Turkey, Bulgaria, etc ...).

2. White asbestos (chrysotile) has minor bio-persistence. Its fibres, spiral and loose over the years, are altered by organic liquids. It is generally accepted that chrysotile asbestos is less carcinogenic than amphibole types. However, it is nearly always contaminated by a small proportion of tremolite, which could confer pathogenicity to the commercial chrysotile (Hodgson and Darnton 2000).

In general, asbestos fibres appears as microscopic needles, whose carcinogenicity depends on bio-persistence, surface properties, and dimensions. The fibres have a thickness of less than 1 µM and a length varying from 3 to 300 µM. The longer ones (> 4 µM), and thin (<0.25 m in diameter) are more dangerous, because of their major capability to passively migrate from the alveoli to the pleural interstitium. Asbestos is particularly carcinogenic when its fibres may be dispersed into the surrounding environment, as a result of any kind of stress: mechanical, wind, thermal stress or run-off rain water.

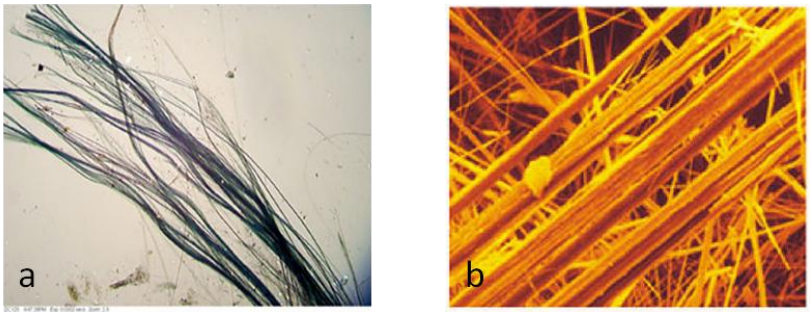

Figure 5: chrysotile (a), and amosite (b)

Asbestos fibres can cause tissue damage trough essentially three mechanisms: ingestion, skin contact, and inhalation. It is the inhalation of the fibres, in a dose which exceeds the body's ability to defend itself, the mechanism more dangerous. The main diseases associated with inhalation of asbestos fibres are asbestosis, pleural plaques, lung cancer, MPM, pleural effusions, and rounded atelectasis.

Normally, the pleura is protected from the inhaled external agents, which are trapped in the alveoli or interstitium. The inhaled particles are removed by phagocytosis and transported by the dense network of lymphatic vessels to the pulmonary hilum. The amphibolic asbestos fibres are able to overcome, exceptionally, the barrier lung-pleura, and the lymphatic drainage of them is difficult.

The exact mechanism by which asbestos fibres migrate to the pleural surface is not known yet. Moreover, it is not possible to explain why the same substance, once in the pleural space, may cause

pleural effusions in some individuals, whereas plaques, pleural fibrosis, or MPM in others. The explanation probably lies in a delicate combination of mechanical, biological, and genetic events.

At the present time, the most accredited hypothesis is that the asbestos fibres accumulate within the tissue and lead to the activation of a chronic inflammatory status of the mesothelium (Donaldson 2010). The inflammation, it is thought, induces mesothelial cells to continuously release reactive oxygen species (ROS), as well as cytokines and growth factors, such as the hepatocyte growth factor (HGF), the epidermal growth factor (EGF), the platelet-derived growth factors (PDGFs) (Lee et al., 2007), and the vascular endothelial growth factor (VEGF) (Pasello and Favaretto 2009). An elevated expression of PDGF (chains A and B) were observed in the early response to asbestos and were found to be mitogens for normal mesothelial cells (Gerwin et al., 1987; Langerak et al., 1996), and to act as paracrine stimulators for the proliferation of the MPM (Metheny-Barlow et al., 2001; Pass and Mew 1996). However, asbestos may also cause auto-phosphorylation of EGFR (Zanella et al., 1996), resulting in activation of mitogen-activated protein (MAP) kinase cascade and phosphorylation of extra cellular signal-regulated kinases 1 and 2 (ERK1/ERK2). Studies of transforming growth factor (TGF)-β in both cell lines and MPM samples have shown the over-expression of three isoforms, including both active and latent forms of TGF-β1 and TGF-β2 (Fitzpatrick et al., 1994).

On the other hand, the asbestos fibres which interact with the alveolar macrophages, are captured and entrapped within lysosomes (Matsuzaki et al., 2012). It seems that this leads to the activation of NLRP3 inflammasome, to cleave procaspase 1 to an active form, followed by the activation of pro-interleukin (IL)-1β, and production of ROS, and reactive nitrogen species (RNS) in the macrophages. The consequential apoptotic death of the alveolar macrophages, and the liberation of various cytokines/chemokines, such as IL-1β, tumor necrosis factor (TNF)-α, macrophage inflammatory protein (MIP)-1/2, monocyte chemo-attractant protein-1, and IL-8, cause chronic inflammation and proliferation of collagenic fibres as well. This mechanism is continuously repeated: indeed, the released silica particles and asbestos fibres from dead alveolar macrophages are recognized by newly nearby macrophages, with the repetition of similar cellular reactions described above (Hamilton et al., 2008, Thakur et al., 2008).

It is current opinion that all these elements push the mesothelium to proliferate (Lee et al., 2007). The continuous exposure to ROS and to proliferative stimuli is a stress for the mesothelial cells and this may lead to long-term DNA damages (Zucali and Giaccone, 2006). It was also suggested that an increased ROS release could be related to the action of Fe-particles present as contaminant on the surface of asbestos fibres, in particular on the amphibolic type (Simeonova and Luster, 1995).

As generally acknowledged, the DNA lesions could be converted into permanent mutations and/or lead to an epigenetic reorganization of the DNA. These events represent among the most important molecular changes for reprogramming cells towards a malignant phenotype.

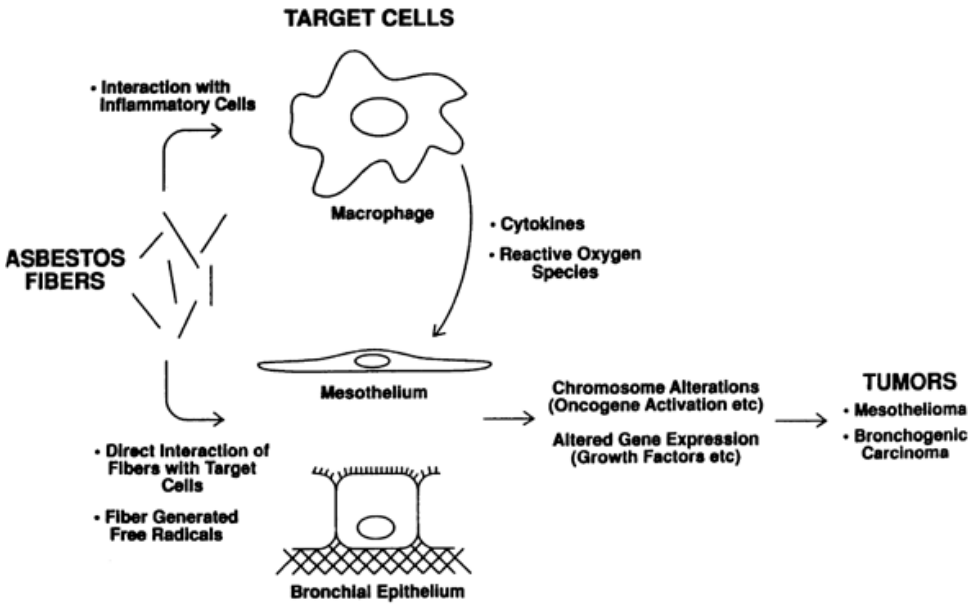

Figure 6: Mechanisms of action of asbestos fibres.

1.5.2 SV40 infection

In addition to asbestos exposure, many investigations have found an association of MPM with the oncogenic simian virus (SV) 40 (Klein et al., 2002; Cristaudo et al., 2005). Indeed, a transforming synergistic action between asbestos fibres and SV40 has been proved in human, hamster, and murine mesothelial cells and in animal models (Pietruska et al., 2007; Robinson et al., 2006; Kroczynska et al., 2006). However, not all the authors agree with this view (Aoe et al., 2006) and the role of SV40 in the aetiology of MPM for humans is still to be ascertained completely. The Simian Virus 40 (SV40) is a DNA virus, found in both monkeys, and humans. The virus was firstly identified by Dr. Eddy in 1964 in cultures of rhesus monkey kidney cells, employed to produce polio vaccine (Eddy, 1964). It has been hypothesized that the virus was transmitted from monkeys to humans mainly in the period 1955-1963, through the administration, worldwide, of anti-polio vaccines contaminated with SV40 (Strickler et al., 1998). However Carbone et al., have suggested also a possible virus transmission directly by person-to-person contact (Powers and Carbone, 2002). Once inside the host, the SV40 produces two proteins: the large T antigen (Tag), and the small t

antigen (tag). Tag has been shown to bind and inactivate both p53 and pRB in mesothelial cells (Carbone et al., 1997; Gazdar et al., 2002), thus stimulating cell proliferation (Testa and Giordano, 2001). Regarding tag protein, it promotes the MAP signalling pathway, inhibiting the protein phosphatase 2A (PP2A), involved in de-phosphorylation of many protein substrates, including regulators of the cell cycle (Rundell and Parakati, 2001). Mazzoni E et al., have found that in 97 MPM serum samples, the prevalence of antibodies against SV40 viral capsid protein antigens is significantly higher (26%, P = 0.043) than in the control group (15%, n = 168). Taken with caution, this result could suggest that new therapeutic/preventive strategies could be also applied with antiviral drugs or vaccines (Mazzoni et al., 2012).

1.5.3 Exposure to Ionizing Radiations

The exposure to ionizing radiations (IR) is hypothesized playing some role in triggering the disease (Zucali and Giaccone 2006; Carbone et al., 2002; Carbone et al., 2006). It was showed that in rats exposed to asbestos and IR the probability to develop MPM is increased, compared to rats exposed only to asbestos (Warren et al., 1981), suggesting a carcinogenic synergistic effect between radiations and asbestos. The MPM is a well-known consequence of radiation therapy used for the treatment of primary malignancy, like lymphoma, breast cancer, lung cancer and other malignancies. Interestingly, most of the reported cases were not previously exposed to asbestos. Teta and collaborators have shown that patients suffering Hodgkin's disease have a 20 times greater risk of developing MPM following radiotherapy (Teta et al., 2007). Although at the time, the role of radiation in the development of MPM remains still unclear, it is possible that IR exposure could act as co-factor with asbestos.

1.5.4 Predisposing Genetic Factors

The individual genetic factors seem to be important in the development of MPM. This has been demonstrated firstly, in a study conducted in three villages of Cappadocia, where erionite, a mineral of the zeolite family, was extracted from quarries, and used as building material. In these villages an “epidemic” of MPM was recorded, and more than 50% of the inhabitants died for the disease (Carbone et al., 2007). Initially, the erionite has been named as the only etiologic factor, but in 2001 Roushdy-Hammady and colleagues investigated whether some families of the villages were genetically predisposed to the development of the malignancy. Through a deep analysis of the pedigree, until the sixth generation (526 individuals in total), the authors highlighted the possibility that MPM was genetically transmitted, as an autosomal dominant disease (Roushdy-Hammady et

al., 2001). The suspicion about a familiarity predisposing to MPM comes also from observations of families such as that reported by Musti and collaborators, on a family with a history of environmental exposure to asbestos. The entire family (mother, father and 4 children) has lived for 13 years in Casale Monferrato, very close to the factory where the father worked, the only one with a real occupational exposure to asbestos. Two sisters have developed MPM, the other one has developed peritoneal mesothelioma, and the brother, pleural plaques, whereas the mother was not affected (Musti et al., 2002). Intriguingly, it is worth to stress that mutations within *BAP1* were reported to be causative for a rare familial form of MPM (Testa et al., 2011). At the present time, this is the only gene identified to confer an increased susceptibility to MPM. Biallelic inactivation of *BAP1* was found in two US families with high incidence of MPM. In one of theme, Testa et al., sequenced *BAP1* in germline DNA identifying that six affected members had identical mutations, whereas unaffected family members did not. In the second analyzed family, germline DNA from three individuals with MPM reported C/G-to-T/A transition in exon 16, creating a stop codon. Moreover the authors have identified that in addition to MPM, some *BAP1* mutation carriers developed uveal melanoma.

Some studies have also suggested that low-penetrant polymorphisms of genes involved in the metabolism of xenobiotics may constitute a risk factor for the development of sporadic MPM.

Associations between risk of MPM, and polymorphisms in specific genes have been reported. Among these, *GSTM1* gene encodes for the enzyme "Glutathione S-transferase", which combines the glutathione to a wide range of electrophilic substances, such as ROS, easily released from the cells and excreted. *GSTM1* is a polymorphic gene, and in some studies it has been shown that the absence of the gene increases the risk of MPM (Hirvonen et al., 1995; Landi et al., 2007). Moreover, for individuals having simultaneously the null genotype for the *GSTM1* gene, and the slow acetylator alleles for *NAT2* gene, the risk of developing MPM doubles, compared to subjects with a present genotype for *GSTM1*, and rapid acetylator for *NAT2* (Hirvonen et al., 1996).

The *NAT2* gene encodes the N-acetyltransferase. The enzyme catalyzes the transfer of the acetyl group from acetyl-CoA on the amino groups. Aromatic and heterocyclic amines are some of its substrates. The response against oxidative stress induced by asbestos fibres, involves products also of other genes, such as manganese superoxide dismutase (MnSOD), one of the most important antioxidant enzymes in mammals (Hayes et al., 2005). It has been shown that the activity of MnSOD is almost absent in normal mesothelial cells of the pleura, while it is higher in MPM cells (Kinnula et al., 1996). The most common polymorphism of MnSOD results in an Alanine (Ala) to Valine (Val) amino acid change at codon 16, producing a conformational change in the protein

secondary structure that may impair the transport of the protein into the mitochondria. (Sutton et al., 2003). Ninety cases of MPM and 395 controls were genotyped using the arrayed-primer extension technique. The Ala/Ala genotypes at codon 16 within MnSOD was showed to be associated with the risk of MPM, and a stronger effect of MnSOD was observed among patients without a clear exposure to asbestos fibres (Landi et al., 2007). Other potentially involved polymorphisms are within the genes involved in DNA repair. In particular *XRCC3* was found associated with MPM. This gene encodes a protein involved in homologous recombination and repair of DNA damage. In a case-control study in Casale Monferrato, variants of *XRCC3* (*XRCC3-241T, XRCC1-399Q*) were associated with an increased risk of MPM (Dianzani et al., 2006). This has been also confirmed by a study with a larger sample size, extended to the city of Turin (Betti et al., 2011).

The studies conducted to date do not clarify in a definitive way what are the susceptibility alleles for MPM. This is also due to the rarity of the tumour, and the consequent difficulty in carrying out studies of case-control with a sample size sufficient to guarantee the desired statistical significance.

1.6 Molecular alterations in MPM

1.6.1 Somatic genetic alterations in MPM

Cytogenetic studies have revealed complex karyotypic changes in the MPM, involving all chromosomes with chromosomal losses more frequent than chromosomal gains. Frequent losses are localized on chromosome arms 1p, 3p, 4q, 6q, 9p, 13q, 14q, and 22q and gains involve chromosome arms 1q, 5p, 7p, 8q, and 17q. (Krismann et al., 2002; Lindholm et al., 2007; Taniguchi et al., 2007; Ivanov et al., 2009; Cheung et al., 2010; Christensen et al., 2010; Jean et al., 2011). Mutations of proto-oncogenes and, deletions, loss of heterozygosity, or inactivation of tumor supressor genes occur frequently in basically all human cancers. Interestingly, mutations within p53 and pRb, among the most frequently mutated genes in cancer, are rare in the MPM (Lee et al., 2007). However, one of the predominant genetic abnormality in the MPM is the homozygous inactivation of the gene *CDKN2A* within the 9p21 region, occurring at a frequency of greater than 70%, (Prins et al., 1998; Chiosea et al., 2008). *CDKN2A* is regulated in order to allow stem cells to proliferate and plays a critical role also in the cellular senescence of mature cells, through the regulation of both the pathways pRb and p53 (Gil and Peters, 2006). The locus encodes two distinct proteins (overlapping genes), $p16^{INK4a}$ and $p14^{ARF}$, originating from different transcription start sites and translated from mRNA undergone to alternative splicing $p16^{INK4a}$ exerts its tumour suppressive effect by inhibiting the cyclin-D-dependent kinases (CDKs), thus preventing the CDK-mediated hyper-phosphorylation and the inactivation of pRB, that normally leads to the cell cycle arrest at the G_1-phase (Zucali and

Giaccone, 2006). $p14^{ARF}$ inactivates the human homolog of mouse double minute 2 (*MDM2*), which is an upstream regulator of p53 (Sekido, 2008).

The TSG neurofibromin 2 (*NF2*), a gene mutated in the type 2 neurofibromatosis (an autosomal dominant hereditary disease characterized by tumours of the nervous system) is a tumour suppressor gene mapped to 22q12.2, a region frequently involved as cytogenetic abnormality in the MPM (Flejter et al., 1989; Hansteen et al., 1993; Taguchi et al., 1993). Interestingly, mutations in *NF2* occur in approximately half of MPM cases (Bianchi et al., 1995; Schipper et al., 2003). Although NF2 disease does not usually occur with MPM (Deguen et al., 1998), the risk of developing MPM may increase if an NF2 patient is exposed to asbestos (Baser et al., 2002), likely because of a potential link between asbestos exposure and the *NF2* inactivation (Fleury-Feith et al., 2003). The loss of *NF2* function has been proposed to be an early event in the MPM (Metcalf et al., 1992).

Another chromosomal alteration involved in MPM is the deletion of 3p21 band. This deleted region carries nine genes including *BAP1*, *PHF7*, *SEMA3G*, *TNNC1*, *NISCH*, *STAB1*, *NT5DC2*, *C3orf78*, and *PBRM1*. Given the role of *BAP1* in the rare familial form of MPM, it is conceivable that an altered activity of *BAP1* could provide a reasonable explanation for the frequent loss of 3p21 in MPM. *BAP1* encodes a nuclear ubiquitin carboxy-terminal hydrolase (UCH), called "BRCA1 associated protein-1", belonging to one of several classes of de-ubiquitinating enzymes. This gene contains binding domains for BRCA1 (113705) and BARD1 (601593), that form a tumor suppressor heterodimeric complex. Ventii KH et al., have shown that *BAP1* exerts its tumor suppressor functions by affecting the cell cycle, speeding the progression through the G_1-S checkpoint, and inducing cell death via a process that has characteristics of both apoptosis and necrosis (Ventii et al., 2008). Bott et al., have analyzed 53 samples of MPM patients, looking for additional genes crucial for MPM pathology, and have found that 12 (23%) of theme showed somatic mutations inactivating the gene *BAP1* (Bott et al., 2011). Yoshikawa and collaborators have analyzed the genomic alterations of *BAP1* in 23 samples of MPM (16 epithelioid and 7 non-epithelioid), finding biallelic alterations of *BAP1* in 14 samples (61%). Seven of these showed homozygous deletions of the *BAP1* (partial or total), five had point mutations (including small deletions), while the other two had homozygous mutations without deletions. Of the 14 samples with mutations in *BAP1*, 13 were epithelioid and only one biphasic. These results reveal that the inactivation of *BAP1* occurs at a very high frequency in patients with epithelioid MPM, and this could be useful for diagnosis of epithelioid type MPM (Yoshikawa et al., 2012).

Recently, the new techniques of micro-arrays and deep-sequencing made possible to detect the somatic mutations occurring in the MPM, and to identify new genes involved in driving the carcinogenetic process. Ivanov et al. (Ivanov et al., 2009), performed Representative Oligonucleotide Microarray Analysis (ROMA) on DNA isolated from tumors of 22 patients, confirming the most frequent gains and deletions in the MPM samples, already detected trough the classical CGH analysis. Moreover, analysis of the minimal common areas of frequent gains and losses identified candidate genes that may be involved in different stages of MPM: *OSM* (22q12.2), *FUS1* and *PL6* (3p21.3), *DNAJA1* (9p21.1) and *CDH2* (18q11.2-q12.3).

Sugarbaker et al., used shotgun pyrosequencing to characterize mutations within transcribed sequences unique to MPMs and not present in control tissues (Sugarbaker et al., 2008). Thus, new genes - plausibly related to oncogenesis - were identified, including *XRCC6* (involved in mediating the repair of DNA double-strand breaks via non-homologous end-joining), *PDZK1IP1* (over-expressed in human carcinomas of diverse origin), *ACTR1A* (associated with transport of p53 to the nucleus, its disruption via mutations could potentially result in p53 inactivation,), and *AVEN* (involved in the apoptotic process). Dong et al. (Dong et al., 2009), demonstrated that the whole-transcriptome shotgun sequencing can be a powerful high-throughput tool for the identification of differentially expressed exon junctions resulting from alternative splicing variants. With this study, they suggested two differentially expressed exon junctions (ACTG2.aAug05 and CDK4.aAug05) to be used to classify MPM and normal tissue samples using the median value as a cut-off.

However, we should be aware that the MPM, as many human cancers, is a biologically heterogeneous disease and the studies carried out at genome level are, likely, too few, and often carried out with insufficient number of samples, in order to provide a clear picture of the molecular events. In summary, the complete pattern of molecular changes will need further confirmation and more studies, integrating knowledge from different type of information in a more systematic approach.

1.6.2 Main molecular pathways involved in the MPM

Numerous molecular pathways involved in the MPM have been studied and identified, including the regulation of cell cycle, apoptosis, cell proliferation, and angiogenesis.

1.6.2.1 Apoptosis and Cell cycle

Apoptosis is a form of cell death essential for development, deletion of damaged cells and the turnover of the cells (Danial and Korsmeyer, 2004). Since 1998 it has been reported that the MPM cell lines are more resistant to apoptosis, than the normal mesothelial cells (Narasimhan et al.,

1998). This may be a possible explanation of why the MPM does not respond to the conventional chemotherapy, usually able to induce the apoptotic process. In MPM, several mechanisms of natural inhibition of apoptosis have been described, including the stabilization of the mitochondrial membrane and the inhibition of caspases, through the IAP activity, a family of "proteins inhibiting the apoptosis" (Gordon et al., 2002; Kleinberg et al., 2007). It has been hypothesized that apoptosis can be induced or inhibited by altering the ratio of expression between pro-apoptotic and anti-apoptotic genes, belonging to the Bcl-2 family (Chresta et al., 1996). Inhibitors of Bcl-xL/Bcl-2 emerge as a new class of compounds for disrupting the balance between pro-apoptotic and the anti-apoptotic stimuli. The exposure of tumor cells to inhibitors of Bcl-2/xL, alone or in combination with other chemotherapeutic, has shown a synergistic inhibition of tumor growth, inducing apoptosis and increasing the chemo-sensitivity both in vitro and in vivo, and it may represent a new therapeutic strategy in the treatment of MPM (Cao et al., 2007).

An important factor involved in the immortality of cancer cells is also the transcription factor NF-kB. Once activated, its p65 subunit translocates to the nucleus to activate IAP. The deriving effect is the promotion of the cells growth and differentiation and, moreover, the induction of the angiogenic factors synthesis. After the activation of TNF-α induced by asbestos, NF-kB increases cell survival, allowing cells with DNA damage, asbestos caused, to proliferate, rather than die. The therapeutic targeting toward the signaling TNF-α/NF-κB may decrease drug resistance and increase cytotoxicity in MPM cells (Carbone and Bedrossian, 2006).

1.6.2.2 Proliferation and angiogenesis

The particular growth factors that affect MPM cell growth are under investigation.

The hepatocyte growth factor / scatter factor (HGF / SF) is a multifunctional growth factor that can induce many biological functions, critical for the malignant phenotype, including invasion, proliferation and morphogenesis. The receptor c-Met is the only known receptor for HGF / SF. Downstream of c-Met, more signalling pathways are activated, including Ras / Erk, PI3K/Akt, and c-Src kinase pathways. It has been demonstrated an autocrine loop of HGF / SF / c-Met in both cell lines and MPM tissue samples, and the over-expression of HGF and c-Met has been associated with increased microvascular density and an increased expression of matrix metalloproteinases (Tolnay et al., 1998).

It has been noticed that the small molecule SU11274, through the inhibition of c-Met, is able to induce the suppression of cell growth in some MPM cell lines, but not in non-malignant mesothelial cells. In particular, SU11274 inhibits cell migration, and the signal transduction induced by HGF (Jagadeeswaran et al., 2006).

The cytoplasmic tyrosine kinase Src is often expressed and activated in MPM. The activity of SRC kinases family is associated with an advanced stage of MPM, and it may contribute to invasiveness and metastatic spread. In preclinical models, the dasatinib, a potent inhibitor of SRC kinases family, prevents the migration and the invasion of MPM (Tsao et al., 2007). In MPM it was also observed the over-expression of EGFR (epidermal growth factor receptor), especially in the epithelioid subtype. An important molecule, involved in EGFR downstream signalling is the phosphoinositide-3-kinase (PI3K), whose pathway appears to be active in MPM cell lines (Cacciotti et al., 2001). The PI3K/AKT pathway is involved in the regulation of cell size, proliferation and survival, as well as in the formation of cancer metastasis, angiogenesis and invasiveness. The PI3K/AKT pathway can be tested as a target for inhibiting the growth of MPM cells, although it has been observed that cell lines of human MPM with high constitutive activity of AKT are more resistant to drugs (Ramos-Nino et al., 2005).

The MPM patients have the highest levels of VEGF (vascular endothelial growth factor) compared to patients suffering of any other solid tumor. Indeed, VEGF, VEGF-C and their receptors are over-expressed in the tissue of MPM, in cell lines and in pleural effusions (Strizzi et al., 2001). In addition, elevated levels of VEGF have been observed in the sera of MPM patients, compared to the serum of normal subjects. In particular, MPM cell lines express high levels of VEGF and its receptors (VEGFR-1 and VEGFR-2), compared with normal mesothelial cells. Neutralizing antibodies against VEGF and VEGFR 1-2 reduce significantly the viability and the proliferation of the MPM cells in vitro, while the pre-treatment in vivo with an antibody anti-VEGFR reduces the malignant pleural effusion in a murine model. VEGF may also act in an functional autocrine loop that directly stimulates the growth of cells of MPM. In fact, the production of VEGF can have an impact on patients survival, not only promoting tumor angiogenesis, but also by directly stimulating tumor growth (Zucali et al., 2011). High levels of VEGF in the pleural were associated with reduced survival in patients with MPM (Strizzi et al., 2001). Similarly, the over-expression of VEGF, as detected by immunohistochemical analysis, predicts a shorter survival in patients with MPM (Demirag et al., 2005). To conclude, the expression of VEGF and VEGF-C in MPM is correlated with microvessel density, and high density of microvessels is associated with poor survival.

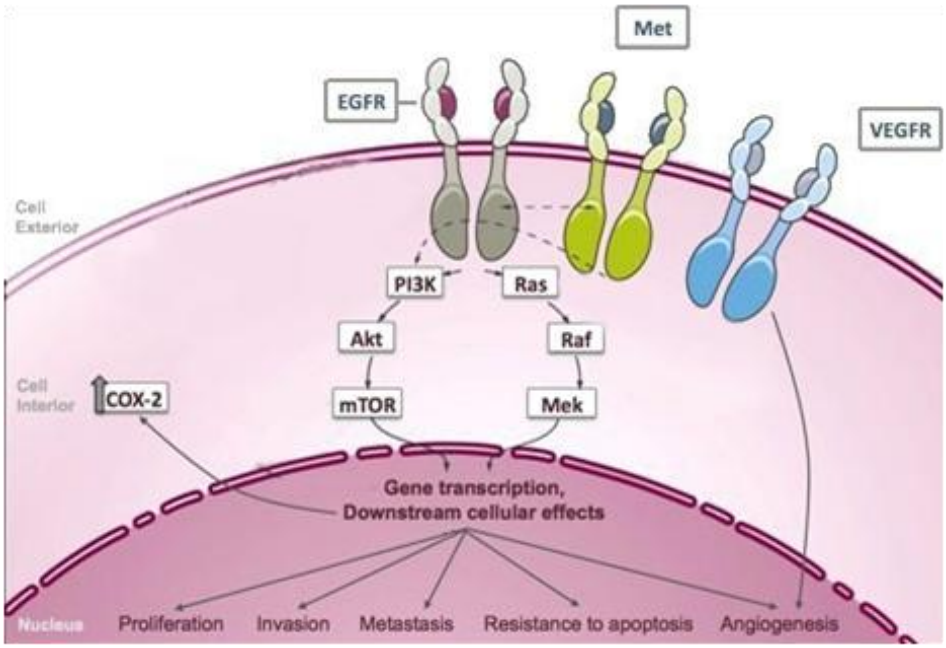

Figure 7: Signaling pathways of EGF, VEGF, and HGF

In recent years it has become clear that the MPM is characterized by activation of survival pathway and inhibition of tumor suppressor (Fennell, 2011). Other molecules have been identified (Fox and Dharmarajan, 2006). Among them, *WNT* (that seems to play an important role in homeostasis and development), mesothelin (regulatingd apoptosis and cancer cell growth) (Wang et al., 2012), and calretinin, whose up-regulation seems to increase resistance to asbestos cytotoxicity and thereby contributing to fibrers carcinogenicity (Henzi et al., 2009).

1.7 Diagnosis of MPM

1.7.1 The importance of an early detection

The MPM is characterized by non-specific clinical signs and symptoms, i.e. pleural effusions, short-breathing, chest pain, lack of appetite, weight loss, and night sweat. For this reason, early diagnosis results very difficult (Melaiu et al., 2012). At the present time, improved detection methods for diagnosis of asymptomatic MPM are lacking, but needed for an early and reliable detection and treatment of this disease. A definitive and early diagnosis on effusion samples could be important, because advances in therapy for patients with MPM may result in an improved outcome if they are applied to stage I disease (Pomjanski et al., 2008). The International Association for the Study of Lung Cancer Staging Committee developed an international database, focusing on patients managed surgically. It has been reported that because of the absence of valid methods with which recognize

promptly the MPM, no significant difference on median survivals (by clinical TNM and pathological TNM) are present: stage I, 21 months; stage II, 19 months; stage III, 16 months; and stage IV, 12 months (Rusch et al., 2012). The need to detect the MPM at the early stages led several authors to investigate whether tumor-related biomarkers can contribute towards the evaluation of the carcinogenic risk in populations exposed to asbestos.

Amati M et al., analyzed 94 asbestos-exposed subjects, 22 patients with MPM, and 54 healthy subjects. They evaluated levels of 8-hydroxy-2'-deoxy-guanosine(80HdG) in white blood cells, and plasma concentrations of soluble mesothelin-related peptides (SMRPs), angiogenic factors, and matrix proteases for potential early detection of MPM. The combination of 80HdG, VEGFβ and SMRPs best distinguished the individual groups (asbestos-exposed subjects, controls and MPM patients), suggesting a potential indicator of early and advanced MPM cancers (Amati et al., 2012).

Recently, Ostroff et al., conducyed a case-controls study, using SOMAmer proteomic technology, which simultaneously measures over 1000 proteins in unfractionated biologic samples, identifying a promising biomarker panel for surveillance and diagnosis of MPM (Ostroff et al., 2012).

To date, specific tests, currently employed in case of suspected MPM are following (Moore et al., 2008):

- Chest X-ray allows to show abnormalities of the pleura or lung.
- Computer Assisted Tomography or Computerized Tomography (CT) is useful to determine the presence of cancer, its exact location, and its possible spread to other organs, thanks to the visualization of the whole pleura surface, and diaphragm, but also of the livers and adrenal glands (Armato et al., 2008). The most significant evidences, allowing the discrimination between MPM and benign pleural disease are: pleural thickening, mediastinal pleural involvement, and circumferential pleural rind. However, the absence of this picture does not exclude the MPM diagnosis (Leung et al., 1990).
- Positron Emission Tomography (PET) allows to measure the metabolic activity of a lesion: the value obtained in case of MPM is significantly higher, compared with that from other benign disease (Wang et al., 2004; Bénard et al., 1998).
- Magnetic Resonance Imaging (MRI) is not so often employed to assess a MPM case. However it can provide additional information above CT, with a more precise identification of the staging of the tumor, and of its extension into the diaphragm (Wang et al., 2004).
- Biopsy: the pleural sample can be excised from the chest (thoracentesis), abdomen (paracentesis) or the cavity around the heart (pericardiocentesis), to verify, under a microscope, the presence of

tumor cells. The analysis of pleural biopsies allows the diagnosis in 98% of patients (Boutin and Rey, 1993). For this reason it is considered the gold standard for the diagnosis of MPM.

To further ascertain the MPM diagnosis, a useful tool routinely employed is the immune-histochemical analysis, easily reproducible and reliable. The histological investigation is important to distinguish MPM from other types of lung diseases. Positive staining for calretinin, WT-1 (Wilms Tumor antigen 1), cytokeratin 5/6, podoplanina, mesothelin, and thrombomodulin; and negative staining for carcinoembryonic antigen (CAE), and for other epithelial antigens, generally expressed by lung adenocarcinoma, such as TTF-1 (Thyroid Trascription Factor 1) (Scherpereel et al., 2010), can address for the MPM diagnosis.

If the MPM is of sarcomatoid or mixed type, the histologic diagnosis is complicated by the combination of epithelioid cells and spindle-shaped. To distinguish the sarcomatoid subtype, from the squamous cell lung carcinoma, antibodies anti-cytokeratins as positive markers, and anti-p63 and anti-desmin, as a negative marker (Scherpereel et al., 2010) are used. However, there is currently no marker that provide 100% specificity and high sensitivity for the diagnosis of MPM.

1.7.2 Calretinin as potential biomarker for MPM

A promising immuno-histochemical marker, which seems highly specific, compared with the others commonly employed, is the calretinin (CALB2). It has been found positive in 97% of the MPM cases, with the majority of them showing both cytoplasmic and nuclear staining (Shield and Koivurinne, 2008). This data has been confirmed also recently by Dinu M et al., (Dinu et al., 2012). CALB2 is a calcium binding protein belonging to the large EF-hand family, involved in the control of the calcium signal function, needed for numerous cellular functions as gene expression, synaptic transmission, cell cycle progression and apoptosis. CALB2 is a 29KDa protein, whose structure is highly conserved. The sequence of 261-271 amino acids is organized into six EF-hand domains. The EF-hand motif is formed from a conserved sequence of amino acids (29–30 residues): an alpha helix, a highly conserved 12 residue sequence that functions as the calcium-binding domain, followed by a second alpha helix oriented perpendicular to the first. Of the six EF-hand motifs present in calretinin, only five are active. Of the active domains, four display high affinity calcium-binding sites with positive cooperativity (Schwaller et al., 1997), while the remaining domain displays lower affinity binding (Stevens and Rogers, 1997).

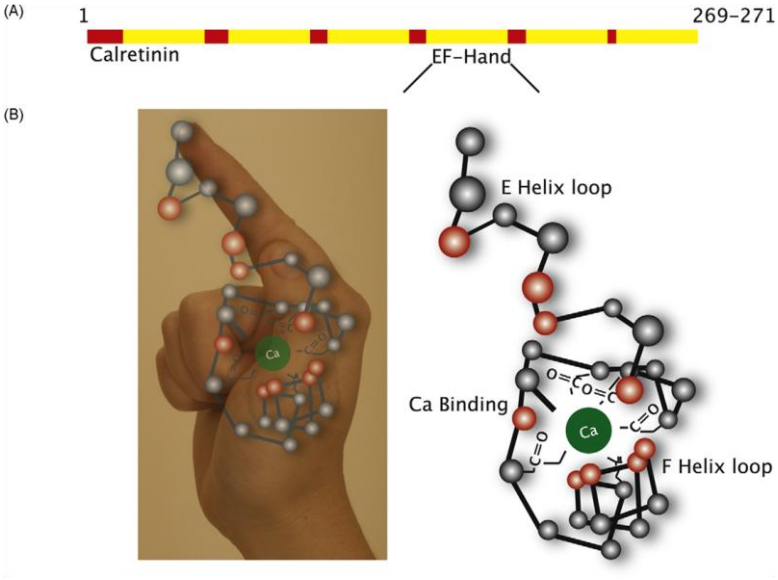

Figure 8: (A) Schematic representation of calretinin. The yellow blocks indicate the E-helix-loop-Fhelix- hand (EF) hand regions. **(B)** The three-dimensional structure of the EF-hand calcium-binding domain can be visualized using the right hand: the index finger represents residues 1–10 (the E-helix), the middle finger represents residues 10–21 (the calcium-binding loop), and the thumb represents residues 19–29. Modified by Camp and Wijesinghe, 2009.

CALB2 is expressed in distinct neuronal populations including the retina, granule cells of the cerebellum, and brainstem auditory neurons. It is also expressed under pathological conditions, particularly in colon adenocarcinoma and epithelial MPM cells. In addition to playing a role in modulating neuronal excitability, *CALB2* has been proposed as target in gene therapeutic approaches. Inase et al., explored the 5-flanking region (2.2 kb) of the calretinin gene as a tumor-specific promoter, showing that 2 MPM cell lines transfected with the pCAL-TK expression vector became about 100 times more sensitive to ganciclovir than the parental cells, and suggesting the calretinin promoter a promising candidate as a specific and efficient promoter in MPM (Inase et al., 2001).

However, an early diagnosis with the described techniques is extremely difficult. The characteristics of the samples themselves do not facilitate the differential diagnosis of pleural diseases: pleural effusion and thoracoscopic biopsies could have several problems such as small size, compression artifacts, and poor fixation. For this reason, the search for tumor markers useful to detect the tumor from the earliest stages of its development is needed.

1.7.3 Mesothelin: diagnostic and prognostic marker in the MPM?

The mesothelin is one of the most promising biomarkers for the surveillance of asbestos-exposed people, as well as for the prognosis and clinical course of MPM (Cristaudo et al., 2011).

Mesothelin (MSLN), a 40 KDa glycosylphosphatidylinositol (GPI) anchored cell surface protein, is normally expressed at low levels in mesothelial cells and over-expressed in several human tumors, including MPM, ovarian and pancreatic adenocarcinoma. Recent studies have shown the expression of mesothelin gene (*MSLN*) also in lung adenocarcinomas (Ho et al., 2007), uterine serous carcinoma (Dainty et al., 2007), acute myeloid leukemia (Steinbach et al., 2007) and cholangiocarcinoma (Ordóñez, 2003; Yu et al., 2010). *MSLN* maps to chromosome 16p13,3, consists of 17 exons, its cDNA is of 2130 bp, and contains an open reading frame of 1884 bp. Human *MSLN* encodes a ~71kDa precursor protein of 622 amino acids, having a hydrophobic sequence at the C-terminal, removed and replaced by a phosphatidylinositol, and with four potential glycosylation sites. Following glycosylation, the precursor is proteolytically cut by a furin-like protein at Arg295 into two products, a ~31kDa mature megakaryocyte potentiating factor (MPF residues Ser34 – Arg286) (Yamaguchi et al., 1994) and a ~40kDa GPI-anchored membrane-bound mature mesothelin starting from Glu296 (Chang and Pastan, 1996; Hassan et al., 2004; Hassan and Ho, 2008).

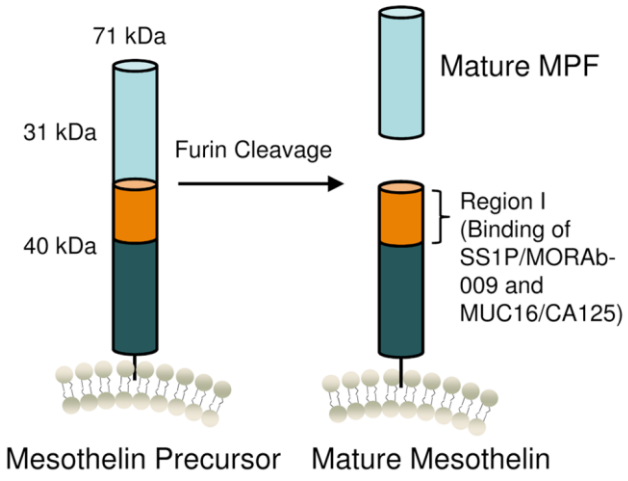

Figure 9: Structure of mesothelin (Tang et al., 2013)

Hassan et al., have reported 2 protein variants of *MSLN* (Hassan et al., 2004). The variant 1 presents an insertion of 8 amino acids, after the glutamine 408 and arises a protein still able to bind to the plasma membrane. The variant 2 maintains the intron between exons 16-17, giving rise to a protein missing the C-terminal, because of an alternative splicing mechanism. This protein isoform is

released from the cell and defined "soluble mesothelin related protein" (SMRP). Immunohistochemical investigations revealed that the main form of MSLN anchored to the membrane is the 40 kDa ones. However it has been found also a glycosylated form of 71 kDa (Hassan et al., 2006). It is not clear which is the variant of MSLN / MPF detected by ELISA in different human tumors.

A full understanding of biological functions of *MSLN* is lacking. It seems that *MSLN* pays a role in cancer progression, cell-adhesion, metastasis, and chemoresistance (Tang et al., 2013).

The link between adhesion molecules and *MSLN* was suggested early, following studies on the ovarian carcinoma. It was suggested that *MSLN* might be involved in the adhesion and spread of ovarian cancer cells throughout the mesothelial lining of the peritoneal cavity (Chang and Pastan, 1996). Moreover, recently, it was clearly shown that deficiency of *MSLN* in Tsc2-KO mice was associated with a decreased number and size of renal tumors, reduced cell proliferation, increased apoptosis, inhibited cell adhesion to collagen-coated plates, and suppressed tumor formation in nude mice. In that study, it was shown that *MSLN* affected the expression of mature integrin b1, thereby affecting also the phosphorylation of several downstream proteins, such as FAK, Akt, rpS6, and Stat3 (Chang and Pastan, 1996). In addition, in pancreatic cancer cells, the over-expression of *MSLN* determines the up-regulation of growth factors such as IL-6, known to be involved in cancer survival/proliferation and tumor progression (Bharadwaj et al., 2011). Conversely, the *MSLN* silencing reduced the IL-6 levels. *MSLN* also induces an increase in NF-κB activation which leads to resistance to TNFα induced apoptosis (Bharadwaj et al., 2011), indicating a mechanism through which *MSLN* may help increase survival of tumour cells in the highly inflammatory milieu, evident in pancreatic cancer through Akt/PI3K/NF-κB activation and IL-6 over-expression. In breast cancer cells it has been shown that the over-expression of *MSLN* promoted anchorage-independent growth in soft agar. Moreover, cells expressing high levels of *MSLN* exhibited resistance to anoikis, a type of apoptosis induced by detachment from substratum, as indicated by decreased DNA fragmentation and down-regulation of the proapoptotic protein Bim (Uehara et al., 2008). Elliot L. Servais et al., have shown that *MSLN* expression promotes MPM cell invasion and metalloproteinases secretion in both human and murine MPM cells. In a tissue microarray from epithelioid MPM patients, *MSLN* over-expression correlated with higher MMP-9 expression at individual core level. According to the authors, this evidence elucidated a biologic role for *MSLN* as a factor promoting tumor invasion and MMP-9 expression in *MSLN*-expressing MPM (Servais et al., 2012). Furthermore, it has been proposed that serum mesothelin (SMRP) could be a sensitive and highly specific biomarker for MPM. Indeed, concentrations of mesothelin in serum of MPM

patients are significantly higher than those of healthy individuals (Beyer et al., 2007). Robinson was the first to understand the potential of mesothelin in the early diagnosis. When the concentration of SMRP was measured in 40 healthy controls previously exposed to asbestos and monitored for 5 years, seven showed high levels of SMRP and 3 of them developed MPM, and one lung cancer. None of the remaining 33 with low levels of mesothelin developed any disease (Robinson et al., 2003). Other research groups have reported elevated levels of SMRP in patients with MPM, compared to subjects previously exposed to asbestos (Scherpereel et al., 2006; Cristaudo et al., 2007; Grigoriu et al., 2007; Creaney et al., 2007; Creaney et al., 2008; Pass et al., 2008; Schneider et al., 2008; Azim et al., 2008; Iwahori et al., 2008; Rodríguez Portal et al., 2009; Davies et al., 2009; Hollevoet et al., 2010; Cristaudo et al., 2011). Furthermore, it has been demonstrated that the SMRP results much higher not only when compared with the levels of mesothelin in healthy pleural controls, but also when compared with other cancers (including lung cancer) or other inflammatory lung or pleural disease (Cristaudo et al., 2007; Creaney et al., 2008; Pass et al., 2008; Schneider et al., 2008; Rodríguez Portal et al., 2009; Hollevoet et al., 2010). Overall, according to various authors, the mesothelin is nowadays considered a promising marker for the epitheliod MPM, but it is less attractive as biomarker for the sarcomatoid type (Beyer et al., 2007; Hollevoet et al., 2010; Grigoriu et al., 2007).

In other studies SMRP has been proposed as marker useful for prognostication and monitoring of treatment response in MPM. Grigoriu BD et al., (Grigoriu et al., 2009) measured the SMRP levels in 40 patients diagnosed with MPM and subjected to gene-transfer therapy or conventional treatment. They have found that in patients, with baseline SMRP levels greater than 1 nM, and disease progression after therapy, the SMRP increased (by 2.1 nM at two, 5.2 nM at four and 1.3 nM at 6 months), whereas patients, who responded to treatment, had an initial small decrease of mesothelin, followed by a return to baseline values after 6 months of follow-up. Wheatley-Price P (Wheatley-Price et al., 2010) collected serial plasma samples from 41 patients with MPM, and observed that seven patients who underwent surgical resection with negative margins had elevated preoperative SMRP levels that fell to normal postoperatively. Moreover, rising SMRP was observed in all patients with radiologic disease progression. In a study in which 91 MPM patients have been enrolled, and SMRP levels measured, it has been observed that changes in mesothelin level were correlated to objective response to chemotherapy, as assessed radiologically and by PET imaging, and with patient survival. In 55 patients who received chemotherapy, change in mesothelin correlated with radiological response ($\chi(2) = 11.32$; $P = 0.023$) and change in metabolically active tumor volume ($r = 0.58$; $P < 0.01$). Median survival for patients with a reduction in mesothelin

following chemotherapy (19 months) was significantly longer than for patients with increased mesothelin [5 months; $P < 0.001$ (Creaney et al., 2011)].

In summary, more studies are needed in order to detect novel deregulated genes and altered molecular pathways in the MPM, useful as biomarkers for a more effective early diagnosis and of prognostic value.
Nowadays, novel methods of investigation are available in order to better ascertain whether a given gene is involved in specific biological process.

1.8 Novel methods for the functional study of individual genes

Among the available methods, the use of siRNAs has been considered a powerful strategy allowing to study the function of any gene. For this reason, nowadays, the use of "RNA-interference" (RNAi) is of increasing interest. This term designs an endogenous mechanism allowing the degradation of specific mRNA. siRNAs are small RNA of about 21-23 nucleotides, that bind their mRNAs target with a perfect complementarity and trigger their destruction (Baselga, 2006; Carthew and Sontheimer, 2009). They could derive from the cleavage mediated by Dicer (an endoribonuclease belonging to the RNaseIII family) of a long non-coding double-stranded RNAs either produced in the cell, introduced by viruses, or also by transfection (Sashital and Doudna, 2010). The RNAi is an evolutionary conserved mechanism and the great interest resides in the fact that, with a simple transfection of a siRNA, it is possible to knock-down the expression of a specific gene. For this reason, this tool has a wide application in basic research (Lindbo et al., 1993; Tamura et al., 2006). Moreover, siRNA have potentially unlimited therapeutic applications and preliminary studies are ongoing (Dykxhoorn, 2009; Lindbo et al., 1993). Indeed, siRNAs have some distinct advantages over conventional drug therapies: they have high specificity, a potentially enormous number of targets, and a fast and common method of synthesis as respect to small molecules (Vaishnaw et al., 2010), as shown in table 2.

	siRNA	Small molecule
Specificity	High, sequence driven	Low-medium, conformation driven
Potency	Typically pM	Variable
Number of accessible targets	>>1000	500 to 1,000
Number of potential leads and backups	>>10 to 100, depending on length of target	<2 to 3
Speed to lead molecule	4 to 8 weeks	2 to 4 years
Species crossreactivity	High	Low
Manufacturing	Common, rapid, scalable methods	Variable, can be complex

Table 2: A comparison of various drug discovery attributes of siRNAs and small molecules (Vaishnaw et al., 2010)

Algorithms have been developed for screening and selection of siRNAs. These are necessary to identify segments of double-stranded 19-23 bp that have the minimal complementarities with the no-target molecules, for the synthesis of siRNA with small size, for in vitro assays in order to assess the power and non-specific cytotoxicity, and for pharmacological evaluating in vivo (Vaishnaw et al., 2010).

In order to better understand the role of genes/pathways in the development of the MPM, in the past few years, gene silencing strategy has been attempted, alone or in combination with the conventional chemotherapy. Several target genes have been proposed to stop the progression of the pleural malignancy. Here some examples: (1) REV3 is the catalytic subunit of DNA polymerase ζ. Inhibition of REV3 expression increases the sensitivity of human MPM cells to a variety of DNA-damaging agents and reduces the formation of resistant cells. (Knobel et al., 2011). (2) ZDHHC8 is a putative palmitoyl-transferase enzyme containing a DHHC domain, proposed as a novel molecular-targeted radiosensitizing agent. Thanks to immunohistochemical analysis, decreased cell proliferation and induction of apoptosis have been observed in tumors treated with ZDHHC8 siRNA and X-irradiation, but not with ZDHHC8 siRNA alone (Sudo et al., 2012). (3) Since it has been suggested that Mcl-1 cooperates with Bcl-x(L) for protection against cell death, Varin E et al., investigated the response of MPM cell lines to the down-regulation of Bcl-x(L) (alone or in combination with cisplatin) and the potential interest of its concomitant inhibition of Mcl-1. Bcl-x(L) depletion sensitized two highly chemoresistant MPM cell lines to cisplatin: one cell line displayed an apoptotic type of cell death, whereas the other evidenced mainly necrotic-type cell death. Moreover, the simultaneous inhibition of Bcl-x(L) and Mcl-1 induced a massive cell death in the absence of chemotherapy (Varin et al., 2010). (4) Data from Mohammed research group

suggested EphA2 as potential target in the MPM therapy. This gene belongs to the ephrin receptor subfamily of the protein-tyrosine kinase family, and it has been shown that silencing of EphA2 cause the induction of both extrinsic and intrinsic apoptotic pathways in MPM cells. Its targeting may thus be an effective approach for inhibiting MPM growth (Mohammed et al., 2011).

In summary, the combination of the attractive mechanism of action, and of the relative ease of manufacturing explain why both biotechnology and pharmaceutical companies have shown great enthusiasm for RNAi therapeutics (Haussecker, 2008). However, these experimental approaches were also very useful in order to determine whether a gene could play an important role for sustaining the malignant phenotype.

2. Aim

Given the difficulty of an early diagnosis, the limited therapeutic approaches now available, and the rapid progression of the MPM, there is the need to understand the molecular mechanisms involved in carcinogenesis and in chemo-resistance. So, in this study we investigated genes potentially deregulated in MPM, as candidate genes involved in the initiation, sustainment and evolution of MPM, with the following purposes:

1) Identification of early diagnostic markers
2) Identification of putative therapeutic markers
3) Gain novel insights into mechanisms related to responses to chemotherapeutic drugs

3. Materials and Methods

3.1 Review of literature data

3.1.1 Data collection

In order to identify a MPM-gene (MG), i.e. a gene crucial for any process leading to the disease (e.g. initiation, maintenance, or clonal evolution), we made an extensive review of the literature. Specifically, assuming that most of the MGs could be differentially expressed in MPM as compared to normal mesothelial cells, we focused on microarray studies of cell transcriptome. In this kind of studies a great number of transcripts are analyzed without formulating any a priori hypothesis. This approach should prevent any bias coming from previously established knowledge leading to an over-representation of specific genes. To systematically collect MPM microarray studies published until 2011, PubMed (URL: http://www.ncbi.nlm.nih.gov/pubmed/) was searched with keywords related to the study background. The search was limited to English-language publications. Only studies where the expression profiles of MPM cells were compared to normal mesothelial cells were included, thus excluding papers with different types of control samples (i.e. different histotypes of MPM). Microarray studies using both MPM derived cell lines and MPM tissue samples were included. Studies carried out on animal models of MPM and studies where MPM cells have been exposed to drugs were excluded. Only a few microarray studies compiled the mentioned criteria. This probably depends on the difficulty of acquiring a large number of tissue samples, due to the low incidence of MPM. Thus, we selected a total of nine papers (listed in Table 3), out of which only four had complete information about microarray experiments and results retrievable from ArrayExpress (http://www.ebi.ac.uk/arrayexpress/) or GEO (http://www.ncbi.nlm.nih.gov/geo/). Due to the small number of selected studies, and the non-availability of raw data for half of them, performing a meta-analysis was not feasible. Each selected study was then thoroughly analyzed, paying particular attention to: (i) the employed microarray platform, (ii) the number and type of samples, (iii) the fold-change, and (iv) the statistical significance of deregulated genes. In gathering this information, we noticed that no uniform criteria exist in describing the used materials and methods or in presenting the microarray results. In fact, some papers disclosed only the number or a reduced list of differentially expressed genes considered interesting by the authors (Crispi et al., 2009), in other cases the information concerning the used microarray platform was only approximate (Gordon et al., 2005) and not all the studies make the raw data available by public repositories. In order to retrieve missing or unclear data, specific information was requested to the

corresponding authors of the manuscripts or to the microarray suppliers. Thus, for each study we recorded the complete platform information (i.e. microarray supplier, design version, the list of genes or ESTs probed on the microarray, etc.) except for one, which used a no longer commercially available platform (MicroMax Human cDNA Microarray System II-TSA; Perkin Elmer Life Sciences; Boston, MA). We finally collected the lists of differentially expressed genes produced by the authors' analysis with the relative fold-changes and statistical significance values. Gene names were uniformed according to the HUGO nomenclature. All this body of information was resumed in the supplementary table S3 of the review (Melaiu et al., 2012), where each gene was identified as up-regulated or down-regulated in the MPM, simply according to what reported by the papers' authors, without any re-interpretation of the results. We only applied a selection threshold, by excluding genes too weakly deregulated, that means with fold changes ranging between 0.7 and 1.3.

References	Microarray platform	Tot. Genes	MPM cell lines	MPM tissue samples	Control cell lines	Control tissue samples
Rihn et al. 2000.	A	3962	1		1	
Kettunen et al. 2001.	B	588	4		2	
Singhal et al. 2003.	C	4132		16		4
Hoang et al. 2004.	D	4800	10	4	1	
Mohr et al.2004.	E	9984	1	2	1	
Gordon et al. 2005.	F	14500	4	40	1	9
Crispi et al. 2009.	G	38500		9		4
Røe et al. 2009.	G	38500		5		6
Røe et al. 2010.	G	38500		6		7

Table 3. Schematic representation of the transcriptome studies evaluated in the present RTS

Legend: A= Incyte Pharmaceuticals arrays (UnigemV, Genome system Inc., St Louis, USA)
B= Atlas Human Cancer gene filters (Clontech)
C= GF211 GeneFilters Microarrays (Research Genetics, Inc., Carlsbad, CA)
D= MicroMax Human cDNA Microarray System II-TSA; Perkin Elmer LifeSciences; Boston, MA
E= Human UnigemV from IncyteGenomics (Palo Alto, CA, USA)
F= Affymetrix Human U133A (Affymetrix, Santa Clara, CA, USA)
G= Affymetrix Human Genome U133 Plus 2.0 GeneChip (Affymetrix, Santa Clara, CA, USA)

3.1.2 Finding a consensus among RTS genes

A first requirement for a gene to be considered a true MG was to appear reproducibly deregulated in independent studies. However, the absolute number of positive calls alone is not exhaustive by itself, unless adjusted to the total number of analyses carried out by different studies. For example, two independent studies showed that *SOD1* and *JUNB* were up-regulated in the MPM (Appendix 1, Melaiu et al., 2012), but while *SOD1* was analyzed in eight different studies, only two studies (both positive) analyzed *JUNB*. Table 10 in the text and Appendix 1 (Melaiu et al., 2012) report the ‘‘total number of studies’’ that analyzed a particular gene and the number of ‘‘positive studies’’ where that gene was found statistically deregulated. Findings from literature were then summarized by writing a U (up-regulated) or D (down-regulated) for each publication, and by specifying the tumor histology when possible. Most of the studies, in fact, either did not mention the tumor histology or merged all the MPMs into a unique histotype. These studies were reported as ‘‘MPM unspecified’’ in the fourth column of Table 10 in the text (and Appendix 1, Melaiu et al., 2012). Because in one study (Hoang et al., 2004) different histotypes were analyzed, the sum of the Us (or Ds) is greater than the total number of positive studies, such as the case of *JUNB*. Genes described as U and D for the same histotype in different studies, were classified as ‘‘contradictory’’ and no further analyzed. Genes resulted as U in a sub-type of MPM, but D in another were classified as ‘‘inconsistent among histotypes’’ and no further analyzed. Both these classifications, together with the full dataset, are reported in the second-last column of Appendix 1. In order to reduce any publication bias due to similar findings from different studies by the same authors, we considered just one paper for authors’ group. Results, corrected by authors, are reported in Table 10 and Appendix 1. In order to rank RTS data, for each gene a score based on the probability values from the binomial distribution was calculated, given by the probability mass function:

$$\text{P-value} = \binom{n}{k} p^k (1-p)^{n-k}$$

This function returns the probability of getting exactly k successes in n trials. Specifically, for each gene the probability to observe k positive studies (i.e. successes), in n independent studies (i.e. the total number of independent studies) was calculated. The a priori probability (p) for an event to occur corresponds to the total number of positive events, divided by the total number of the analyses.

3.2 Data mining

To confirm results obtained by RTS, an automated search in literature by using the following data mining (DM) tools: SNPs3D (URL: http://www.snps3d.org/), GeneProspector (URL: http://hugenavigator.-net/HuGENavigator/geneProspectorStartPage.do), and Coremine (URL: http://www.coremine.com/medical/) was performed. Each of the selected tools adopts a different algorithm to mine information from a text or a database and connect gene or protein names to keywords of interest. Furthermore, the level of confidence that each tool expresses on the achieved results is based on different scoring or statistical methods. In order to find a group of as exhaustive as possible keywords, both the Coremine ability to connect each other Medical Subject Heading subcategories relating to MPM, and SNPs3D filter of MPM aliases were used. The lists of keywords were inspected for consistency with MPM by using Medical Subject Heading Terms and the information from the studies' background. The following list of keywords was used to interrogate the tools: Pleural Neoplasms, Malignant Pleural Mesothelioma, Pleural Mesothelioma, Asbestos Exposure, Cisplatin, Cisplatin Treatment, Cisplatin Resistence, mpm, malignant pleural, mpm cells, mpms, mpm cell, mpm patients, mpm cell lines, pleural mesotheliomas, pleural malignant, malignant pleural mesothelioma mpm, asbestos-exposed, cisplatin, cisplatin treatment, cisplatin resistance, asbestos. A binomial distribution was applied to associate a statistically significant value to each gene correlated to MPM, based on the total number of retrieved genes and the number of tools which found each gene. The gene lists from RTS and DM were compared to look for the degree of overlap.

3.3 Gene expression analysis

3.3.1 Tissues collection

Twenty fresh-frozen MPM and 20 non-MPM pleural tissues were collected, thanks to the collaboration with the surgeons of the Cardio-thoracic Department of the hospital "Cisanello" of Pisa (Italy) and with the Occupational Medicine Unit of the Hospital "Santa Chiara" of Pisa. MPM were surgically resected during the pleurectomy/decortication procedures and the specimens were eye-inspected by pathologists to minimize the collection of non-cancerous tissues. Control tissues were normal pleura from patients diagnosed for lung cancer undergone to surgery (6 diagnosed for lung adenocarcinoma and 14 for lung squamous cell carcinoma). Similarly as before, the specimens

were eye-inspected, in order to collect a small portion of pleura not containing signs of lung cancer spread. All samples were immediately placed in 50mL Falcon tubes containing ice-cold RNA*later* (Qiagen, S.p.A, Milano, Italy), a RNA preservation solution, and stored at −80◦C until use. The collection of MPM resulted in 14 MPM of epithelioid type, 3 sarcomatoid, and 3 biphasic. Diagnoses were all confirmed by the Pathological Anatomy unit of the hospital, following the standard clinical routine. All patients gave informed consent for the research and the local ethical committee approved the study, according to the Helsinki declaration.

3.3.2 Cell cultures

Four mesothelioma cell lines (Mero-14, Mero-25, IstMes2, and NCI-H28) and one mesothelial non-MPM immortalized cell line (MeT-5A) were used. Mero-14, Mero-25, and IstMes2 MPM cells have been kindly donated by the Istituto Tumori of Genova (National Research Council, Genova, Italy). The MeT-5A mesothelial cells, and the NCI-H28 MPM cells were purchased from the ATCC (American Type Culture Collection) and kindly donated by collaborators of the Pharmaceutical Department of the University of Pisa. Met-5A, Mero-14, Mero-25, and NCI-H28 cell lines were verified for their identity, by analysing the genetic markers reported in the certification. IstMes2is a cell line established locally and no certification is available. Mero-14, Mero-25, and IstMes2 were cultured in DMEM medium (Lonza, Basel, Switzerland), supplemented with 10% fetal bovine serum (Sigma Aldrich Corp. St Louis, MO, USA) and 1% Pen-Strep (Lonza, Basel, Switzerland). The NCI-H28 cell line was grown in RPMI 1640 medium (Gibco, Life Technologies, Monza, Italy), supplemented with 10% fetal bovine serum (Sigma Aldrich Corp. St Louis, MO, USA) and 1% Pen-Strep (Lonza, Basel, Switzerland. The MeT-5A cell line was grown in Medium199 with HEPES (Life Technologies, Monza, Italy) supplemented with 3.3 nM epidermal growth factor (EGF, Life Technologies, Monza, Italy), 400 nM hydrocortisone (Sigma Aldrich Corp. St Louis, MO, USA), 870 nM insulin (Life Technologies, Monza, Italy), 10% fetal bovine serum (Sigma Aldrich Corp. St Louis, MO, USA), and 1% Pen-Strep (Lonza, Basel, Switzerland). All of them were maintained at 37°C in a 5% CO_2–humidified atmosphere (Forma* 311 Direct Heat CO2 Incubator, Thermo Scientific, Waltham, MA, USA).

3.3.3 RNA isolation and cDNA synthesis

Total RNA was isolated from individual tissue samples by using Tri-Reagent (Sigma Aldrich Corp. St Louis, MO, USA) according to the standard protocol. Sample was first grinded in a mortar, then mixed and homogenized with 0.7 ml Tri-Reagent using the electric homogenizer TissueRuptor

(Qiagen, S.p.A, Milano, Italy). To ensure complete dissociation of nucleoprotein complexes, the sample was allowed to stand for 5 minutes at room temperature before adding 0.2 ml of chloroform. The mixture was shaken and left at room temperature for 10 min and centrifuged at 12,000 x g for 15 min at 4^{o}C. The chloroform completes the denaturation of proteins and, thanks to its high density, facilitates the separation of the aqueous phase colorless, containing the RNA, from the organic phase, containing DNA, lipids and proteins. At the interface between the two phases, there are the denatured proteins. The upper aqueous phase was transferred to another fresh centrifuge tube and RNA was precipitated with 0.5 ml of isopropanol. After being incubated at room temperature for 10 min, the sample was centrifuged at 12,000 x g for 10 min at 4^{o}C to get the RNA pellet, which was subsequently washed by 75% (v/v) ethanol. Centrifugation was then performed and the RNA pellet was air-dried and resuspended in 25 μl of RNAse free water. In order to remove possible contaminating genomic DNA, the extracted RNA was treated with DNase buffer (Sigma Aldrich Corp. St Louis, MO, USA). Concentration of clean-up RNA was determined spectrophotometrically (SmartSpec 3000, Bio-Rad Laboratories, Hercules, CA) at 260nm. The ratio of readings at 260 and 280 nm (A260/A280) provided an estimate of the purity of RNA with respect to contaminants that absorb in the UV spectrum, such as proteins and DNA. The integrity and purity of total RNA was further verified by electrophoresis on ethidium bromide agarose gel, inspecting the 18 and 28S ribosomal RNA bands. Whether the absorption ratio was outside the window encompassing 1.9 - 2.1, or any other quality control criterion was not met, the extraction was repeated. The reverse transcription (RT) of mRNA to cDNA was performed using the *iSCRIPT cDNA Synthesis Kit* (Bio-Rad Laboratories, Hercules, CA). cDNA synthesis was carried out from 1 μg of total RNA in a final reaction volume of 20 μl. The reaction is catalyzed by the Moloney Murine Leukemia Virus (MMuLV) reverse transcriptase, an RNA/DNA-dependent DNA polymerase modified and optimized for the synthesis of cDNA, in presence of an mRNA-polyA template and a single-stranded oligo-dT.

3.3.4 Selection of candidate genes

From the previously elaboration of data published on the MPM (described above), we selected for validation 77 genes, on the basis of a probability value taking into account, for each gene, the number of studies with positive findings (i.e. deregulated in a statistically significant way) as well as the number of negative studies. These genes were either well-known markers of cell transformation, or involved in cell proliferation, cell cycle checkpoints, immortalization, cell adhesion, or known to play role in cancer progression.

3.3.5 Primer design and quantitative Real-Time PCR (QPCR)

For each gene, primer pairs were designed using the publicly available web-based tool *Primer3* (http://frodo.wi.mit.edu/), and the software "Beacon Designer" (PREMIER Biosoft), whereas the analysis of the oligonucleotides was performed with the online tool "Netprimer" (PREMIER Biosoft) available at the URL: *www.netprimer.com*. Primer pairs were designed to allow a specific amplification of the spliced mRNA, avoiding any possible amplification of the genomic DNA. To this aim, most of the primers were designed on the exon-exon boundaries or on exons spaced by long intronic sequences (i.e. >2000 base pairs). Primers were tested for their specificity to have unique hits within the whole genome, with "Primer-BLAST" algorithm within BLAST webpage, available at the URL: www.ncbi.nlm.nih.gov/tools/primer-blast. Each PCR was tested using melting curve analysis to verify the presence of gene-specific peaks and the low abundance of primer-dimers. The PCR products were also run on agarose gel electrophoresis in order to test further the specificity of the amplicons, following eye-inspection.

To assess the efficiency of each primer pair, five serial dilutions were prepared from a pool of cDNAs and used as template for the generation of the standard curves. QPCR was set up using 2 µl first-strand cDNA template, 16 µl deionized H_2O, 0.3 µM of upstream and downstream primers and 5 µl 5× Eva Green master mix with ROX as reference dye (Solis, Tartu, Estonia). The thermal cycling conditions were 15 min at 95°C followed by 15 s at 95 °C, 30 s 60°C (40 cycles) and 30 s at 65 °C. Based on the Ct values for all dilution points in a series, a standard curve was generated using linear regression analysis. The PCR amplification efficiency of each primer pair is calculated from the slope of a standard curve. To ensure reproducibility of the experiments, all the reactions were executed in triplicate and the average was used for the statistical analyses.

When the primer pair did not meet the required criteria of specificity or showed a low efficiency (thresholds: $r^2>0.96$, $3.11<slope<3.58$), TaqMan probes (Life Technologies, Monza, Italy) were employed. For the TaqMan assay, the reaction mixture consisted of 2 µl of cDNA template, 7 µl of deionized H_2O, 1 µl of specific TaqMan Assay probe and primers mixture, and 10 µl of TaqMan® Gene Expression Master Mix (Life Technologies, Monza, Italy). The thermal cycling conditions were: 15 min at 95°C followed by 15 s at 95 °C, (40 cycles) and 60 s at 60 °C. Primers and TaqMan assays are reported in tables 4 and 5 respectively.

Gene Symbol (HUGO)	**NM**	**Primers sequences (*foward*/*reverse*)**	**T_m (°C)**	**Amplicon lenght**
CCND2	NM_001759	gCT ggC TAA gAT CAC CAA CAC A CCT CAA TCT gCT CCT ggC AA	60	62 bp
ITGA6	NM_000210	gCT ggT TAT AAT CCT TCA ATA TCA ATT gT TTg ggC TCA gAA CCT Tgg TTT	60	113 bp
TIMP3	NM_000362	CCA ggA CgC CTT CTg CAA C CCT CCT TTA CCA gCT TCT TCC C	60	71 bp
AKR1C1	NM_001353	ggA TTA TgT TgA CCT CTA CCT TAT T TTT TTC CAT TTT CAT CTT TTg ggA T	60	86 bp
CDKN1A	NM_001220778	ggC AgA CCA gCA TgA CAg ATT gCg gAT TAg ggC TTC CTC TT	60	73 bp
IGFBP4	NM_001552	CCC ACT CCC AAA gCT CAg ACT CCA AgC AgA Tgg TgC AAC AA	60	89 bp
ASS1	NM_000050	ATT gAC ATC gTg gAg AAC Cg gCC TCg ATg TCT AAA TgA gCA	60	101 bp
BUB1B	NM_001211	gTg CTT CCC AgT TTC ACT CC CCA ggC TTT CTg gTg CTT Ag	60	110 bp
ITGA4	NM_000885	AgA TgC Agg ATC ggA AAg AA CCC CAA CCA CTg ATT gTC TC	60	117 bp
NUSAP1	NM_016359	gCC AAg AgT CTg ggT CTC C TCA TTT CCT TTT CTT gCC TCA	60	95 bp
CFB	NM_001710	Tgg AAA ACC Tgg AAg ATg TTT ggT TgC TTg Tgg TAA TCg gT	60	109 bp
BLMH	NM_000386	AAT TCA Tgg ggT gAA gAC CA CTg TCC ACC ACC ACT TCg TA	60	92 bp
CDK2AP1	NM_004642	TCT TAC AAA CCg AAC TTg gC ggC ggT ACT gTg AAg ACg TT	60	106 bp
COL6A1	NM_001848	AgA ggA gAC CCT ggT gAA gC Cgg TAg CCT TTA ggT CCg AT	60	110 bp
CRIP1	NM_001311	ATg CCC AAg TgT CCC AAg T CAg CgT CTT CCC ACA TTT CT	60	114 bp
VCAN	NM_004385	TCT CCC CAG GAA ACT TAC GA CAC TCT TTT GCA GCC TCC TC	60	110 bp
DAB2	NM_001343	AAg TCA TgC TCg CTT CAC g CCA gTg gAC ACT Tgg TgA CA	60	108 bp
EEF2	NM_001961	CTg gAg ATC TgC CTg AAg gA gCA CgT TCg ACT CTT CAC Tg	60	109 bp
IARS	NM_013417	TCA CCA gAg gAT CTT CCC TT CAC CTT TTg gAT TTT CCA gg	60	111 bp
NME2	NM_001018137	AAA gAC CgA CCA TTC TTC CC ATC ACT Cgg CCT gTC TTC AC	60	104 bp
PDGFRB	NM_002609	AAC TgT gCC CAC ACC AgA Ag CAg gAg AgA CAg CAA CAg CA	60	106 bp
RAN	NM_006325	CAg gAg AAA TTC ggT ggA CT ATg CCA gTT Agg CAC ATT CTT	60	111 bp
RCN2	NM_002902	Tgg ATg ATg CAg AAg Agg Ag TTC AAg ACT CAA ACC ggg AC	60	101 bp
THBS1	NM_003246	CAA TgC CAC AgT TCC TgA Tg CAC AgC TCg TAg AAC Agg Agg	60	110 bp
THBS2	NM_003247	TCA ggg gTT TgC TTC AgA AC gTT CTC ACT gAT ggC gTT gA	60	116 bp
TNPO2	NM_001136195	gAg Tgg gTg gTC AAg gAg TC ggA TCA gCT CAg gCA ggT Ag	60	94 bp

CXADR	NM_001338	ATT TCg CCA gAA gTT TgA gTA TCA TgC ATg gCA gAT Agg CAg TTT	60	81 bp
PCNA	NM_002592	Agg CAC TCA Agg ACC TCA TCA gAg TCC ATg CTC TgC Agg TTT	60	76 bp
RAD21	NM_006265	ggA AAg AgA CAg gAg gAg TA gCg TgT AAA gAg CTT CAg TA	60	86 bp
SOD1	NM_000454	Agg CCC CTT AAC TCA TCT CTA CAg gTA CTT TAA AgC AAC TCT	60	122 bp
SPINT2	NM_021102	CAA gTg CTC CCA gAA ggC CgT Tgg Cgg TgC AgT ATT C	60	78 bp
CALB2	NM_001740	TAT GGA AGC ACT TTG ACG CA GAC ATC ATG CCA GAG CCT TT	60	106 bp
ALDOA	NM_000034	Cgg gAA gAA ggA gAA CCT g gAC CgC TCg gAg TgT ACT TT	60	98 bp
DSP	NM_004415	CTC AAT CAg CAT CCA gCT TC gAA CAT CAA TgC ACT Tgg Tg	60	100 bp
PGK1	NM_000291	CAA gCT ggA CgT TAA Agg gA CTT ggg ACA gCA gCC TTA AT	60	108 bp
PGM1	NM_001172818	CCg ACT gAA gAT CCg TAT TgA T gAA CgC AgT TAA CTg CCg Ag	60	110 bp
VEGFA	NM_001025366	CTA CCT CCA CCA TgC CAA gT AgC TgC gCT gAT AgA CAT CC	60	104 bp
SFRP2	NM_003013	CAA CgA CAT AAT ggA AAC gC Tgg TCT TgC TCT Tgg TCT CC	60	116 bp
NR3C1	NM_001018077	TTC CCT ggT CgA ACA gTT TT TgT TgT TgC TgT TgA ggA gC	60	96 bp
LGALS3BP	NM_005567	CAT gAg TgT ggA TgC TgA gT CAg CTT gTg gAA gCA CTT g	60	109 bp
SSBP1	NM_ 001256510	ATA ATg TgA ggC gAC AAg CA Tgg CCA AAg AAg AAT CAT CC	60	103 bp
SYNE1	NM_ 182961	ggg ATA TCg CCA ATg TgA Tg ggC CAg ATg AgA gTT gAT CC	60	92 bp
UPK1B	NM_006952	CTA CCg TgT gCg CAg AAA Tgg AAg CAA CgA ACA gTT gA	60	92 bp
FGF2	NM_002006	ggA gAA gAg CgA CCC TCA C AgC CAg gTA ACg gTT AgC AC	60	94 bp
CCNH	NM_ 001199189	TCT gTT gTg ggT ACg gCT Tg AgT gAg CAT TAT TAT CCT ggg g	60	90 bp
PTGS2	NM_000963	CAT gAT gTT TgC ATT CTT TgC TgA TTT AAg TCC ACC CCA Tg	60	111 bp
COL1A1	NM_000088	AAg Agg AAg gCC AAg TCg Ag CAC ACg TCT Cgg TCA Tgg TA	61.4	91 bp
FEN1	NM_004111	Agg gAg AgC gAg CTT Agg AC ggC AAC ACA gAg gAg ggA T	60	104 bp
HEG1	NM_020733	CAg CgT TAC CTA gTT ACA TC AAT AgC gTC ACA TTg gAg	60	109 bp
FGF9	NM_002010	gTg gAC TCT ACC TCg ggA Tg CCA gTT TTC TTC gAA CTg TTC TC	60	98 bp
MCM4	NM_005914	ggC AgA CAC CAC ACA CAg TT CgA ATA ggC ACA gCT CgA TA	60	108 bp
GALNT7	NM_017423	AgT ggT CCT CTg gTC TTC CC gCA Tgg ggT CAT TgA CAT CT	60	92 bp
TMEM176A	NM_018487	ggA TTT TTC TAC ATC CgC gA gTA CCA CCC CgT TTC TCg TA	60	119 bp

Table 4. Selected candidate target genes, primers, and amplicon length.

Gene Name (HUGO)	NM	Assay ID	T_m (°C)	Amplicon lenght
SULF1	NM_001128205	Hs00290918_m1	60	65 bp
TOP2A	NM_001067	Hs03063307_m1	60	72 bp
CCNO	NM_021147	Hs00221731_m1	60	79 bp
FANCI	NM_001113378	Hs00383049_m1	60	66 bp
CHEK1	NM_001114122	Hs00967506_m1	60	79 bp
PECAM1	NM_000442	Hs00169777_m1	60	65 bp
SMARCA4	NM_003072	Hs00231324_m1	60	106 bp
KRT5	NM_000424	Hs00361185_m1	60	133 bp
KRT18	NM_000224	Hs01653110_s1	60	81 bp
ASPA	NM_000049	Hs00163703_m1	60	63 bp
C10orf116	NM_006829	Hs00428992_m1	60	89 bp
DAP	NM_004394	Hs00234397_m1	60	102 bp
EID1	NM_014335	Hs00534885_s1	60	58 bp
FAS	NM_000043	Hs00236330_m1	60	125 bp
METAP1	NM_015143	Hs00299385_m1	60	61 bp
PDGFRA	NM_006206	Hs00998018_m1	60	84 bp
RHOB	NM_004040	Hs03676562_s1	60	82 bp
CENPF	NM_016343	Hs01118845_m1	60	77 bp
MSLN	NM_005823	Hs00245879_m1	60	65 bp
ANXA4	NM_001153	Hs00984874_m1	60	94 bp
MCM2	NM_004526	Hs01091564_m1	60	67 bp
PLK2	NM_006622	Hs00198320_m1	60	75 bp

Table 5. Selected candidate target genes, TaqMan assay, and amplicon lenght.

3.3.6 GeNorm software and Selection of RT-qPCR reference genes

For a precise comparison of mRNA transcription in different samples or tissues it is crucial to choose the appropriate reference genes. The optimal reference gene should be constantly transcribed in all types of cells at any time of the cell cycle and of the ontogenesis. To identify the ideal group of RT-qPCR reference genes, geNorm (URL: http:// medgen.ugent.be/genorm/), a software that ranks the candidate reference genes based on their stability parameter M, has been employed, according to the *MIQE* guidelines (Bustin et al., 2009). After a literature search to identify the housekeeping genes commonly used in MPM studies, the departing list of reference genes has been analyze in RT-qPCR: *GAPDH*, *HPRT1*, *B2M*, *RPLP0*, *TBP*, *GUSB* and *PPIA* (Tables 6, 7).

Gene Symbol (HUGO)	NM	Primers sequences (*foward*/*reverse*)	T_m (°C)	Amplicon lenght
PPIA	NM_021130	ACT gAg Tgg TTg gAT ggC AAg	60	378 bp
		TCA ACA CTC TTA ACT CAA ACg Agg A		
GAPDH	NM_002046	CCA CTC CTC CAC CTT TgA Cg	60	252 bp
		TgT gAg gAg ggg AgA TTC AgT g		
B2M	NM_004048	CTC CgT ggC CTT AgC TgT g	60	69 bp
		TTT ggA gTA CgC Tgg ATA gCC T		
GUSB	NM_000181	CCT gCC TAT CTg TAT TCA TTg	60	80 bp
		ggg AgT gTg TAg AAg TCA		
TBP	NM_003194	gCg gTT TgC TgC ggT AAT C	57	109 bp
		TCT ggA CTg TTC TTC ACT CTT gg		
HPRT	NM_000194	TTC Agg Cgg CTg CgA CgA g	62	110 bp
		Tgg Cgg AgC AgA ggA ggA g		
RPLP0	NM_053275	CCT CAT ATC Cgg ggg AAT gTg	60	95 bp
		gCA gCA gCT ggC ACC TTA TTg		

Table 6. Selected candidate reference genes, primers, and amplicon length.

Gene Name (HUGO)	NM	Assay ID	T_m (°C)	Amplicon lenght
PPIA	NM_021130	Hs99999904_m1	60	98 bp
GAPDH	NM_002046	Hs99999905_m1	60	122 bp
B2M	NM_004048	Hs00984230_m1	60	81 bp
GUSB	NM_000181	Hs00939627_m1	60	96 bp
TBP	NM_003194	Hs00427620_m1	60	91 bp
HPRT	NM_000194	Hs01003267_m1	60	72 bp
RPLP0	NM_053275	Hs99999902_m1	60	105 bp

Table 7. Selected candidate reference genes, TaqMan assay, and amplicon lenght.

Each of them has been analyzed by using qPCR. In order to analyze the gene expression stability (Vandesompele et al., 2002), Ct values of all samples were exported to Excel, ordered for use in geNorm software and transformed to relative quantities using the gene-specific PCR amplification efficiency (Hellemans et al., 2007), according to the formula:

$$Q = E^{\Delta Ct} = E^{(minCt\text{-}sampleCt)}$$

where E is the amplification efficiency (2 = 100%; 1.95 = 95%); minCt is the lowest average of the Ct, which corresponds to the highest level of expression; sampleCt is the average of the Ct of each

sample. Once the relative quantities have been inserted in geNorm software, the program calculates the M value, and based on that, we can establish the most stable reference genes. Based on the principle that the expression ratio of two ideal reference genes should be identical in all samples, independent of the treatment, condition, or tissue type, increasing variations in the expression ratio between two genes correspond to lower expression stability across samples. geNorm calculates the stability using a pairwise comparison model (Vandesompele et al., 2002) and determines the level of pairwise variation for each reference gene with all other reference genes as the standard deviation of the logarithmically transformed expression ratios. In this way, the reference gene expression stability measure (M value) was calculated as the average pairwise variation of a particular gene with all other control genes included in the analysis (Vandesompele et al., 2002; Maroufi et al., 2010). Lower M values represent higher expression stabilities, and vice versa. geNorm was also used to estimate the normalization factor (NFn) by calculating the geometric mean of the expression levels of the n best reference genes (Vandesompele et al., 2002). Thanks to geNorm, it is also possible to determine the optimal number of reference genes: the optimization of the number of reference genes starts with the inclusion of the two genes with the lowest M value, and continues by sequentially adding genes with increasing values of M. Thus, geNorm calculates the pairwise variation Vn/Vn+1 between two sequential normalization factors NFn and NFn+1 containing an increasing number of reference genes (Vandesompele et al., 2002). A large variation means that the added gene has a significant effect on the normalization and should preferably be included for calculation of a reliable normalization factor. According to the geNorm, if Vn/Vn+1 <0.15 the inclusion of an additional reference gene is not required and the recommended number of reference genes is given by n (Vandesompele et al., 2002).

3.3.7 Normalization and Statistical analysis methods

With the relative quantification, after having transformed into quantities the mean-Ct of the various target genes, it is needed to normalize these values compared to those of reference genes. For each sample the normalization factor has been calculated, according to the formula:

$$NF = \sqrt[n]{REF_1 \times REF_2 \times \ldots \times REF_n}$$

where n is the number of selected reference genes, while REF indicates the Quantity of each reference gene obtained for each sample. The following passage is to divide the quantity of the target gene (for each sample), for the NF of each sample:

$$Q_{sampleX}/NF_{sampleX}$$

The statistical test employed for the comparison of the normalized results is the One-tailed Wilcoxon test. One-tailed Wilcoxon signed rank test was applied to evaluate the statistical significance of the results adopting a threshold of 0.05. The *a priori* hypothesis tested for each gene was derived from the up- or down-regulation identified in the literature review (Melaiu et al., 2012), reported in Appendix 1 (Table S2, Melaiu et al., 2012). The q-value (Storey JD, 2002) was used to correct for multiple testing.

3.4 Mutation screening of *PDGFRB*

3.4.1 Samples collection

Paraffined embedded MPM tissues from 100 surgical resection were available thanks to the collaboration with Prof. Gabriella Fontanini (Pathologic anatomy, Hospital of Pisa, Italy). Informed consent was obtained from all individuals analyzed.

3.4.2 DNA sequencing

To measure the frequency of activating mutations within *PDGFRB* in MPM at the presentation, exons 12 and 18 have been screened. Genomic DNAs were prepared from each sample according to the standard procedure, using the QIAamp DNA FFPE Tissue Kit (Qiagen S.p.A. Milan Italy). The whole exons 12 and 18 of *PDGFRB* were PCR-amplified from genomic DNA using the specific primer pairs reported in Table 8, and sequenced using either PCR oligonucleotide as sequencing primer. The mutation screening was performed with automatic sequencing (Sanger reaction), thanks to the collaboration with Prof. Gabriella Fontanini (Pathologic anatomy, Hospital of Pisa, Italy). With this method, we could detect all the occurring somatic mutations within exons 12 and 18, namely the classical D850 mutation (*PDGFRB*) as well as other eventual mutations in the neighbor sequence encoding for the kinase activation loop domain. Moreover, we could detect the eventual presence of the "gatekeeper" mutation or any other mutations in the neighbor sequence.

Gene Symbol (HUGO)	Exon	Primers sequences (*foward*/*reverse*)	Amplicon lenght
PDGFRB	Exon 12	TgT CCT AgA Cgg ACg AAC CT	258 bp
		CCA ACT TgA gTC CCC ACA CT	
PDGFRB	Exon 18	gAA ggg TCT TTC CCC ACA AT	259 bp
		CAC ACT ggT CAg gAg ggA AT	

Table 8. *PDGFRB*, exons, primers, and amplicon length.

3.4.3 Cell viability for chemoresistance

In order to ascertain whether *PDGFRB* plays a role in the secondary resistance to imatinib, Mero-14 over-expressing *PDGFRB* cells were employed, after having checked the absence of mutations within the target gene, before the treatment with imatinib. Mero-14 cells were seeded onto 96-well plates and allowed to attach 24 h before drug treatment. To assess the IC50, the cells were treated for 48h with different concentrations of imatinib. At the end of the experiment, the [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium], inner salt (MTS) assay was performed using a commercial kit (CellTiter Aqueous Solution, Promega), according to the manufacturer's instructions;. The absorbance values were recorded at 490/620 nm by a spectrophotometric plate reader (Sirio S, SEAC, Florence, Italy) and corrected by subtraction of the absorbance of MTS alone.

3.4.4 Selection of imatinib-resistant MPM cells

Selection of resistant cells was achieved by treating Mero-14 cells to a scaling concentrations of imatinib, starting with a concentration of 25μM and increasing gradually, by 25%, until the concentration of 100 μM, that revealed to be the IC50. Viable cells were grown in the presence of imatinib, replacing the medium twice a week, for about 6 months. Once reached the concentration of 100 μM, viable cells stopped to replicate. At the end of this selection period, genomic DNA was extracted according to the standard protocol, with QIAamp DNA Mini Kit (Qiagen S.p.A. Milan Italy) and processed for next-generation sequencing (NGS).

3.4.5 Samples preparation and NGS

We screened the resistant clones for mutations in exons 12, 14, and 18 of *PDGFRB* by using NGS. The whole exons 12, 14 and 18 of *PDGFRB* were PCR-amplified from genomic DNA using specific primer pair showed in Table 9.

Gene	**Exon**	**Primers sequences (*foward*/*reverse*)**	**Amplicon lenght**
PDGFRB	Exon 12	**CGTATCGCCTCCCTCGCGCCATCAG**TgT CCT AgA Cgg ACg AAC CT	308 bp
		CTATGCGCCTTGCCAGCCCGCTCAGCCA ACT TgA gTC CCC ACA CT	
PDGFRB	Exon 14	**CGTATCGCCTCCCTCGCGCCATCAG**gAg gCC TCC ATA ggg ACT gT	309 bp
		CTATGCGCCTTGCCAGCCCGCTCAGgCT CCT CAg gTA TCC CAA AgA	
PDGFRB	Exon 18	**CGTATCGCCTCCCTCGCGCCATCAG**gAA ggg TCT TTC CCC ACA AT	310 bp
		CTATGCGCCTTGCCAGCCCGCTCAGCAC ACT ggT CAg gAg ggA AT	

Table 9. *PDGGRB*, exons, primers, and amplicon lenght.

The 5'-portion is a 25-mer whose sequence is dictated by the requirements of the 454 Sequencing System for hybridizing to the DNA Capture Beads (Lib-A), and for annealing the emPCR Amplification Primers and the Sequencing Primer; in addition, this 5'-part must always end with the sequencing key "TCAG" used for Amplicon sequencing. The 3'-portion of each primer is designed to anneal with a specific sequence on either side of the target of interest on the DNA sample, delineating the margins of the amplicon that will be produced. This requires detailed knowledge of the target sequence, in particular the sites targeted by the primers.

We prepared 3 different mixes for each primer pairs in order to amplify the three target exons. The protocol employed was:

Primers (10 µM): 0.5 µl F + 0.5 µl R

Buffer BD (10X): 2 µl

MgCl2 (25mM): 1.2 µl

dNTPs (2mM): 2 µl

H2O: 10,6 µl

Hotfire® Taq polimerasi: 0,2 µl

DNA: 3 µl (30 ng/ µl)

The thermal cycle program used included:

95 °C for 8 min (to active the Hotfire® Taq polimerasi)

20 cycles 95°C for 30 sec (to denature the DNA)
68°C for 30 sec, -1°C/cycles (hybridization of primers)
72°C for 1 min and 30 sec (the stage of extension)

20 cycles 95°C for 30 sec (to denature the DNA)
51°C for 30 sec (hybridization of primers)
72°C for 1 min and 30 sec (the stage of extension)

72°C for 10 min (to complete the synthesis)
4°C forever

The visualization of the PCR products was carried out through electrophoresis in 2% agarose gel, obtained through the polymerization of 5 g of agar in 250 ml TBE 0,5X (TRIS borat-EDTA buffer), with the addition of 8 μl of Ethidium Bromide (10mg/ml), at 180V for 20 minutes.
The purified PCR products have been sent to the BMR centre (Bio Molecular Research, CRIBI, Padova, Italy), to perform sequencing through the 454 Sequencing System and the subsequently data analysis.

3.5 Functional studies on *MSLN* and *CALB2* genes

3.5.1 Chemicals

Imatinib was purchased from Cayman Chemical (Michigan, USA); Gemcitabine was obtained from Sigma Aldrich Corp. (St Louis, MO, USA); Cisplatin has been kindly donated by Prof. Justin Stebbing (Imperial college, London). The drugs were dissolved in DMSO at the final concentration of 10 mM. The antibodies used are the following: MSLN mouse monoclonal (Santa Cruz); CALB2 mouse monoclonal (Santa Cruz); β-actin mouse monoclonal (Abcam), p53 mouse monoclonal (Santa Cruz); pERK, mouse polyclonal (Abcam); PARP rabbit polyclonal (Cell Signaling); pAKT rabbit polyclonal (Abcam); ERK1-2 rabbit polyclonal (Abcam); Secondary HRP (horseradish peroxidase)-conjugated goat anti-rabbit IgG and goat antimouse IgG antibodies were from GE

Healthcare. The expression plasmid pcDNA3.1 encoding for *MSLN* (aa 360-2230) was kindly donated by Dr. Chen (Taiwan); the empty vector pcDNA3.1, employed as control, was donated by Dr. Giamas (Imperial college, London).

3.5.2 siRNA transfection

siRNAs were purchased from Qiagen (Qiagen, S.p.A, Milano, Italy). The target sequence for Hs-MSLN-1 (SI0081704) was: CTGGACGTCCTAAAGCATAAA. The target sequence for Hs_CALB2_5 (SI02660980) was: GACGGAAATGGGTATATTGAA. AllStars Negative Control siRNA (SI03650318) was used as non-targeting control. siRNA oligo was re-suspended in the provided buffer at final stock concentration of 20 μM. siRNA transfection was performed with the HiPerfect transfection reagent (Qiagen, S.p.A, Milano, Italy), according to the manufacturer's instruction. In brief, the appropriate number of cells was seeded in different plates. The day after, siRNA was diluted in culture medium without serum, and x μl of HiPerFect Transfection Reagent was added. After an incubation of 10 min at room temperature, allowing the formation of transfection complexes, the mixture was added drop-wise onto the cells. The cells were incubated with the transfection complexes under their normal growth conditions for 6–72 h, depending on experimental setup. Following siRNA transfection experiment, the efficiency of the knock-down of the targeted mRNA was checked.

3.5.3 Plasmid transfection

NCI-H28 cells were employed for the transient over-expression of *MSLN*. Transfections were performed using the FuGENE® Transfection Reagent (Promega Corp. Madison, Wisc., USA) in penicillin/streptomicine-free RPMI for 24 h according to manufacturer instructions. The amounts of FuGENE® Transfection Reagent used were 6 μL for 2 μg plasmid in 95% confluent cells plated onto 6 cm dish (approximately 8 x 10^5 cells/well). pcDNA3 and pcDNA3-MSLN were used, as previously mentioned.

3.5.4 Proteins extraction and Western Blotting

Cell pellets were re-suspended in ice-cold RIPA buffer containing proteases and phosphatases inhibitors. The extracts were then clarified by centrifugation at 15000 rpm for 15 min at 4 °C, and the proteins concentration was determined. Lysates were incubated in 5x sodium dodecyl sulfate (SDS) sample buffer (5 min, 95 °C). 10-30 μg of proteins for each sample was loaded onto 8–15% SDS polyacrylamide gel. Proteins were then transferred onto a nitrocellulose membrane. The

membrane was blocked for 1 hour with non-fat dry milk in TBS containing 0.05% Tween 20, washed, and successively incubated with different primary antibodies for 12 h at 4 °C. The membranes were then washed three times for 10 min and incubated with the HRP-conjugated secondary antibody for 1 h at room temperature (RT). After a thorough washing, the blot was exposed to ECL (GE Healthcare, NJ) and autoradiography. The intensity of the bands was quantified using Image J software (NIH, Bethesda, MD).

3.5.5 Sulphorhodamine (SRB) assay

The SRB assay was performed to test the proliferation of the cells after the depletion of *MSLN* and *CALB2* with and without the combination with 3 different drugs (Imatinib 25 uM; Cisplatin 5 uM; Gemcitabine 1 uM; Imatinib 5 uM + Gemcitabine 1 uM). 3 x 10^3 cells were seeded in a 96 well plate. The day after, one plate was assessed at this time (day 0) and further plates were tested at intervals of 2-d for a total of 6-8 days. Cells were fixed with 100 µL per well of ice-cold 40% (vol/vol) TCA (Sigma Aldrich Corp. St Louis, MO, USA), by gently adding on top of the medium overlaying the cells. The plates were then incubated for 60 min at 4°C. Wells were rinsed five times with tap water and then they were stained with 0.4% SRB solution (100 µl stain/well; Sigma Aldrich Corp. St Louis, MO, USA) for 30 min at room temperature. After staining, SRB solution was poured off, unbound dye was removed by washing five times with 1% acetic acid solution and left to air dry. The bound SRB dye was then solubilized by adding unbuffered Tris-base solution (100 µl/well), and plates were placed on a plate shaker for 10 min at room temperature. Plates were then read at OD 492 nm, using a microplate reader. Every condition was done in quadruplicate. Mean and SEM of independent experiments were calculated. Statistical analysis was performed using a two-tailed Student's *t*-test to determine the statistical significance of the differences observed. A P-value below 0.05 was considered significant.

3.5.6 3D Overlay Culture on Matrigel

Thawed Matrigel (BD Bioscience) in a volume of 70 µL/well was added into each of the wells of the eight-well glass slide chambers (Thermo Scientific), and spread to form a 1-mm thick bed. Matrigel was left to solidify at 37 °C for 15 min. Then, cells (1 × 10^3/well) were plated in medium containing 2% Matrigel and allowed to grow in a 5% CO_2 humidified incubator at 37 °C. Each condition was represented in triplicate.

3.5.7 Flow cytometry (FACS)

To evaluate the effect of the silencing of *MSLN* and *CALB2* on cell cycle progression, Mero-14 cells (subject to the depletion of *MSLN*) and IstMes2 cells (subject to the depletion of *CALB2*) were treated with CT siRNA or *MSLN*/*CALB2* siRNA for 72 h, with and without the combination with the drugs (Imatinib 25 uM; Cisplatin 5 uM; Gemcitabine 1 uM; Imatinib 5 uM + Gemcitabine 1 uM). After the treatment, cells were collected by trypsinization, washed in phosphate-buffered saline (PBS), pelleted by centrifugation and fixed in 70% ethanol. Immediately prior to staining, cells were washed twice in PBS and resuspended in PBS containing 50 μg/ml of RNAse A (Qiagen, S.p.A, Milano, Italy). Cells were stained with propidium iodide (final concentration 100 μg/ml) for at least 1hr at 4°C and were analyzed using a LSR II flow cytometer (BD Biosciences). The percentage of cells in subG1 G_0/G_1, S and G_2/M phases were determined from >10,000 cells using the FACSDiva 6.0 software (BD Biosciences). Three independent experiments were performed for each condition.

3.5.8 Caspase - Glo® 3/7 assay

Caspase-3/7 activation was measured using the Caspase-Glo 3/7 Luminescence Assay (Promega Corp. Madison, Wisc., USA) according to the manufacturer's instructions. Briefly, 3 x 10^5 cells were incubated in a 6 well plate and allowed to adhere overnight. The day after, cells were treated with 40 nM of *MSLN* siRNA, or 50 nM of *CALB2* siRNA (and the CT siRNA), with and without the combination with the drugs (Imatinib 25 uM; Cisplatin 5 uM; Gemcitabine 1 uM; Imatinib 5 uM + Gemcitabine 1 uM), for 48 hr. Afterwards, cells were collected by trypsinization, and approximately 15 x 10^3 cells were transferred in a 96-well white plate. Caspase-3/7-Glo reagent was added, and the samples were incubated at 37°C for 1 h. The luminescence that is proportional to the caspase 3/7 activities was determined by luminometer (Tecan Sunrise, Austria GMBH). Three independent experiments were performed for each condition.

3.5.9 Transwell Cell Invasion Assay

To assess the cell invasion capacity after depletion of *MSLN* and *CALB2*, 5×10^4 cells in 200 μL of α-MEM (Gibco, Life Technologies, Monza, Italy) were plated in the Matrigel-coated upper chambers of the 24-well Transwell invasion assay plate (Corning, NY 14831 USA). Each condition was represented in triplicate. Plates were incubated at 37 °C for 48 h for Mero-14 and IstMes2 cells. Cells in the lower chamber (including those attached to the under surface of the membrane) were

fixed in 4% paraformaldehyde (VWR, Milan Italy), stained with DAPI (Lonza, Basel, Switzerland), and counted with fluorescence microscope (Metamorph – Axiovert microscope).

3.5.10 Wound-Healing Assay

Mero-14 and IstMes2 cells were seeded in a 12-well plate at 25 X 10^3 cells per well. The following day, they were respectively transfected with *MSLN* (40 nM) and *CALB2* (50 nM) siRNAs. After 52 hr, a linear scratch in the confluent cell monolayer was made with a sterile pipette tip. Cells were rinsed and then incubated in full medium 20 hr prior to fixation followed by staining with crystal violet solution to enhance contrast. For each well, pictures were taken at 10× magnification along the scratch area with a phase-contrast microscope.

4. Results

4.1 Review of literature data

4.1.1 RTS

Our main requirement for a gene to be an MG is to be reproducibly deregulated among independent studies. To get this information, and rank RTS data, a binomial distribution has been applied. Since the a priori probability (p) for an event to occur corresponds to the total number of positive events, divided by the total number of the analyses, we have calculated respectively the sum of all the positive findings, corresponding to 1081, and the sum of all the genes on all the platforms used for all the studies, corresponding to 153,383. Thus, 7.05 $x10^{-3}$ was the a priori probability to observe one positive event (a success), that means the probability for each gene to appear as deregulated in a single trial. The P-values are reported in ascending order in the last column of Table 10 in the text and Appendix 1 (Melaiu et al., 2012). Nine hundreds and thirty-one out of a total of 968 identified genes were statistically significant (P-value < 0.05) (see Appendix 1).

4.1.2 Data mining

The DM analysis collected a total of 3236 genes (Melaiu et al., 2012). Coremine detected 3149 genes (CM genes), out of which 657 showed a P-value < 0.05 (CM-significant, CMS), whereas SNPs3D (S3D) and GeneProspector (GP) retrieved 186 and 242 genes, respectively. Based on binomial distribution, 56 (reported in Table 11) out of the 3236 genes were found by all the three tools (P-value = 0.000512), 230 by only two tools (P-value = 0.017664), and 2950 by only one tool (P-value = 0.203136).

4.1.3 Comparison between RTS and DM

RTS and DM results shared the following numbers of genes: RTS vs CM = 387 genes, RTS vs CMS = 84, RTS vs GP = 42, RTS vs S3D = 39, RTS vs (CM + GP + S3D) = 397, and RTS vs (CMS + GP + S3D) = 131, as the Venn Diagram shows (Figure 10).

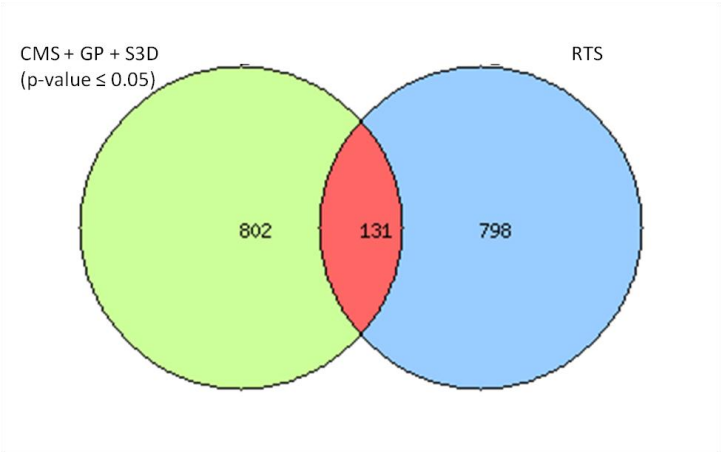

Figure 10: Venn Diagram showing the intersection between RTS and (CMS + GP + S3D), highlighting 131 potential MGs.

DM retrieved about 43% of genes collected by RTS. Genes detected by RTS and not by DM are genes differentially expressed in MPM but never discussed among the sources inspected by DM. In fact, only the most relevant findings of microarray studies are usually reported in literature. RTS was focused specifically on genes detected by microarrays not taking into account genes associated to MPM via different mechanisms (including promoters' methylation, somatic mutations, chromosomal translocations, gene deletions, miRNA regulations and so on). It is not therefore surprising that DM was able to highlight a greater number of genes. However, comparing information from RTS and DM was useful to highlight RTS genes supported by other scientific evidence.

Gene name	Rank	Total number of studies	Total number of studies with a statistically significant deregulation	MPM unspecified	MPM epithelioid	MPM sarcomatoid	MPM biphasic	Total number of studies corrected by authors	Total number of studies with a statistically significant deregulation, corrected by authors	Final judgment	P-value
ANXA4	*1*	6	3	UUU				6	3	Up-regulated	6.85E-06
ASS1	*2*	7	3	UUU				7	3	Up-regulated	1.19E-05
JUNB	*3*	2	2	U	U	U		2	2	Up-regulated	4.97E-05
S100A11	*3*	2	2	UU				2	2	Up-regulated	4.97E-05
PTGS2	*3*	2	2	DD				2	2	Down-regulated	4.97E-05
TMEM176A	*3*	2	2	UU				2	2	Up-regulated	4.97E-05
HEG1	*3*	2	2	UU				2	2	Up-regulated	4.97E-05
RCN2	*3*	2	2	UU				2	2	Up-regulated	4.97E-05
KIF23	*3*	3	3	UUU				2	2	Up-regulated	4.97E-05
PLK2	*10*	3	2	UU				3	2	Up-regulated	1.48E-04
UBXN4	*10*	3	2	U	U	U		3	2	Up-regulated	1.48E-04
DSP	*10*	3	2	UU				3	2	Up-regulated	1.48E-04
EEF2	*10*	3	2	UU				3	2	Up-regulated	1.48E-04
FGF9	*10*	3	2	UU				3	2	Up-regulated	1.48E-04
HELLS	*10*	3	2	UU				3	2	Up-regulated	1.48E-04
SULF1	*10*	3	2	UU				3	2	Up-regulated	1.48E-04
KIF14	*10*	3	2	UU				3	2	Up-regulated	1.48E-04
NMU	*10*	3	2	UU				3	2	Up-regulated	1.48E-04
CENPF	*10*	4	3	UUU				3	2	Up-regulated	1.48E-04
TOP2A	*10*	4	3	UUU				3	2	Up-regulated	1.48E-04
BUB1	*10*	4	3	UUU				3	2	Up-regulated	1.48E-04
CDK2AP1	*10*	4	3	UUU				3	2	Up-regulated	1.48E-04
RAD21	*10*	4	3	UUU				3	2	Up-regulated	1.48E-04
SSBP1	*10*	4	3	UUU				3	2	Up-regulated	1.48E-04
MCM2	*10*	4	3	UUU				3	2	Up-regulated	1.48E-04
FGF2	*26*	4	2	U	U	U		4	2	Up-regulated	2.94E-04
SMC4	*26*	4	2	UU				4	2	Up-regulated	2.94E-04
CDC2	*26*	5	3	UUU				4	2	Up-regulated	2.94E-04
ACSL1	*26*	4	2	DD				4	2	Down-regulated	2.94E-04
AURKA	*26*	5	3	UUU				4	2	Up-regulated	2.94E-04

BIRC5	*26*	5	3	UUU	4	2	Up-regulated	2.94E-04
MKI67	*26*	4	2	UU	4	2	Up-regulated	2.94E-04
AOC3	*26*	4	2	DD	4	2	Down-regulated	2.94E-04
PPARA	*26*	4	2	DD	4	2	Down-regulated	2.94E-04
TGFBR2	*26*	4	2	DD	4	2	Down-regulated	2.94E-04
TSPAN7	*26*	4	2	DD	4	2	Down-regulated	2.94E-04
CCNO	*26*	4	2	UU	4	2	Up-regulated	2.94E-04
FANCI	*26*	4	2	UU	4	2	Up-regulated	2.94E-04
RAN	*26*	5	3	UUU	4	2	Up-regulated	2.94E-04
CCNB2	*26*	5	3	UUU	4	2	Up-regulated	2.94E-04
SMARCA4	*41*	5	2	UU	5	2	Up-regulated	4.86E-04
PTMS	*41*	6	3	UUU	5	2	Up-regulated	4.86E-04
CHEK1	*41*	5	2	UU	5	2	Up-regulated	4.86E-04
CAV1	*41*	5	2	DD	5	2	Down-regulated	4.86E-04
NR4A2	*41*	5	2	DD	5	2	Down-regulated	4.86E-04
PECAM1	*41*	5	2	DD	5	2	Down-regulated	4.86E-04
SFRP1	*41*	5	2	DD	5	2	Down-regulated	4.86E-04
TACC1	*41*	5	2	DD	5	2	Down-regulated	4.86E-04
VWF	*41*	5	2	DD	5	2	Down-regulated	4.86E-04
IFITM1	*41*	5	2	UU	5	2	Up-regulated	4.86E-04
LGALS3BP	*41*	5	2	UU	5	2	Up-regulated	4.86E-04
PTGIS	*41*	5	2	UU	5	2	Up-regulated	4.86E-04
S100A10	*41*	5	2	UU	5	2	Up-regulated	4.86E-04
XPOT	*41*	5	2	UU	5	2	Up-regulated	4.86E-04
NME2	*41*	6	3	UUU	5	2	Up-regulated	4.86E-04
COL1A1	*56*	6	2	UU	6	2	Up-regulated	7.24E-04
CDK4	*56*	6	2	UU	6	2	Up-regulated	7.24E-04
CCNB1	*56*	7	3	UUU	6	2	Up-regulated	7.24E-04
EGR2	*56*	6	2	DD	6	2	Down-regulated	7.24E-04
EGR3	*56*	6	2	DD	6	2	Down-regulated	7.24E-04
EPAS1	*56*	6	2	DD	6	2	Down-regulated	7.24E-04
ALDOA	*56*	6	2	UU	6	2	Up-regulated	7.24E-04
CFB	*56*	6	2	UU	6	2	Up-regulated	7.24E-04
CCT2	*56*	6	2	UU	6	2	Up-regulated	7.24E-04
CDH11	*56*	6	2	UU	6	2	Up-regulated	7.24E-04
COL6A1	*56*	6	2	UU	6	2	Up-regulated	7.24E-04
CRIP1	*56*	6	2	UU	6	2	Up-regulated	7.24E-04

EIF4G1	*56*	6	2	UU	6	2	Up-regulated	7.24E-04
FHL1	*56*	6	2	UU	6	2	Up-regulated	7.24E-04
KIF5B	*56*	6	2	UU	6	2	Up-regulated	7.24E-04
MSLN	*56*	6	2	UU	6	2	Up-regulated	7.24E-04
PGM1	*56*	6	2	UU	6	2	Up-regulated	7.24E-04
PKM2	*56*	6	2	UU	6	2	Up-regulated	7.24E-04
PSMD11	*56*	6	2	UU	6	2	Up-regulated	7.24E-04
VCAN	*56*	6	2	UU	6	2	Up-regulated	7.24E-04
CDK7	*56*	7	3	UUU	6	2	Up-regulated	7.24E-04
PCNA	*56*	7	3	UUU	6	2	Up-regulated	7.24E-04
GAPDH	*56*	6	2	UU	6	2	Up-regulated	7.24E-04

Table 10. Genes showing repeated citations in independent studies (ranked by increasing P-values). U= up-regulated; D=down-regulated.

Gene Names			
ABCB1	*BRCA1*	*FAS*	*PTGS2*
ABCC1	*CASP3*	*FASLG*	*RRM1*
ABCC2	*CASP7*	*GCLM*	*SERPINA1*
ABCC3	*CASP9*	*GPX1*	*SLC28A1*
ABCC4	*CAT*	*GSTM1*	*SOD2*
ABCC5	*CDKN1A*	*GSTP1*	*TNF*
ABCG2	*CDKN2A*	*IFNG*	*TP53*
AKT1	*CYP1A1*	*IL10*	*TP73*
AKT2	*EPO*	*KRAS*	*VEGFA*
ATM	*ERBB2*	*MGMT*	*XPA*
ATP7B	*ERCC1*	*MLH1*	*XPC*
BID	*ERCC2*	*MSH2*	*XRCC1*
BIRC5	*ERCC4*	*NAT2*	*XRCC2*
BRAF	*ESR1*	*NR1I2*	*XRCC3*

Table 11. Genes detected by all the three employed DM tools (Coremine, SNPs3D, GeneProspector). All the listed genes show a P-value of 5.12×10^{-4} from Coremine.

4.2 Gene expression analysis

4.2.1 Specificity and efficiency of primers pairs

The agarose gel electrophoresis (example shown in figure 11) revealed that all primer pairs amplified a single PCR product with expected size, ranging between 90 and 110 bp, as small size amplicons guarantee the best reaction efficiency.

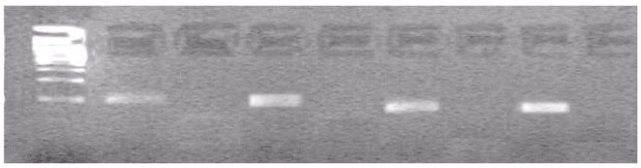

Figure 11: Analysis of primers pairs specificity

The results of the averaged amplification efficiencies for the candidate target and reference genes are shown in table 12 and 13, respectively. The amplification efficiencies ranged between 90.1% and 110%, in agreement with the *MIQE* guidelines.

Gene Symbol (HUGO)	Amplification efficiency (%)	r^2	Gene Symbol (HUGO)	Amplification efficiency (%)	r^2	Gene Symbol (HUGO)	Amplification efficiency (%)	r^2
COL1A1	99.4	0.97	*NME2*	94.9	0.98	*LGALS3BP*	104.8	0.97
HEG1	102.7	0.99	*CFB*	90.1	0.99	*PGK1*	95.4	0.97
FEN1	110	0.99	*SOD1*	98	0.99	*CCNH*	110	0.99
SPINT2	110	0.96	*CXADR*	96.5	0.98	*CDK2AP1*	106.2	0.97
NUSAP1	110	0.99	*UPK1B*	96.8	0.99	*RCN2*	98.6	0.98
CALB2	99.6	0.98	*PCNA*	90.8	0.98	*PTGS2*	92.5	0.99
ASS1	91.1	0.98	*ALDOA*	97.9	0.98	*SYNE1*	96.8	0.99
MCM4	105.9	0.99	*THBS1*	99.4	0.99	*NR3C1*	104.3	0.98
TIMP3	108.8	0.99	*BUB1B*	94.7	0.97	*FGF2*	106.4	0.99
DSP	110	0.97	*SSBP1*	94.5	0.99	*CCND2*	98.9	0.99
TNPO2	107.3	0.99	*COL6A1*	110	0.99	*SARP1*	96.2	0.99
GALNT7	110	0.96	*EEF2*	94.3	0.99	*DAB2*	98	0.99
TMEM176A	110	0.99	*RAD21*	97.4	0.96	*IARS*	101.9	0.99
THBS2	103.1	0.99	*CRIP1*	93	0.99	*CDKN1A*	92.4	0.97
ITGA4	107.3	0.98	*FGF9*	104.1	0.97	*IGFBP4*	98.7	0.99
AKR1C1	105.2	0.98	*VCAN*	108	0.98	*ITGA6*	99.3	0.99
RAN	94.3	0.99	*VEGFA*	110	0.99	*BLMH*	107.5	0.99
PDGFRB	110	0.99	*PGM1*	90.4	0.99			

Table 12: Efficiency of target genes and corresponding correlation coefficient (r^2)

Gene Symbol (HUGO)	Amplification efficiency (%)	r^2
PPIA	104.3	0.996
GAPDH	99.8	0.999
B2M	104.4	0.997
GUSB	103.7	0.992
TBP	110	0.992
HPRT	109.7	0.997
RPLP0	96.3	0.993

Table 13: Efficiency of reference genes and corresponding r^2

4.2.2 Identification of optimal reference genes

For a precise comparison of mRNA transcription in different samples or tissues it is crucial to choose the appropriate reference genes. In our study, geNorm software showed that *RPLP0, HPRT,* and *TBP* were the most stable genes in MPM tissues with M value equal to 1.62, 1.62, and 1.66 respectively (Figure 12).

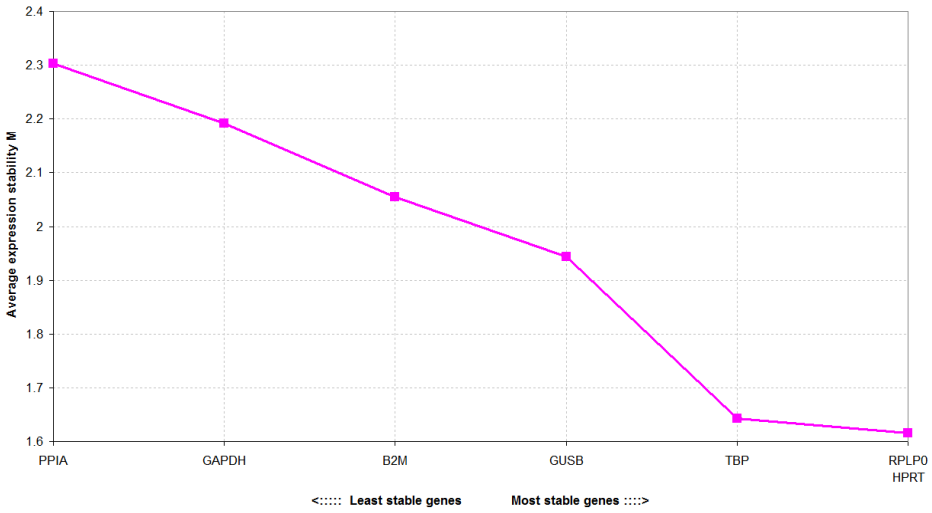

Figure 12: Average expression stability M for the 7 reference genes analyzed

4.2.3 Expression levels of selected genes target

Since we observed heterogeneity among studies and also a biological heterogeneity among tumors, in order to ascertain the reproducibility of the findings obtained from different publications, we have undertaken a validation study to verify the up- and down-regulation of genes that, from our research, are potentially involved in the development and progression of the disease. We analyzed a total of 77 genes. Among them, 53 RT-qPCR showed a correct PCR efficiency (90-110%). The

remaining 24 genes, whose amplification efficiency was not acceptable with the Eva Green RT-qPCR, have been analyzed through the TaqMan® assay, whose efficiency is guaranteed to be 100%. We have analyzed 33 genes detected in both RTS and DM, with high statistical significant level for DM (Table 14), 15 genes with low significant level in DM (Table 15), whereas 29 only found with the RTS (Table 16).

Among the total analyzed genes, 36 were found differentially expressed (DE) in a statistically significant way (q-value <0.05) in tissues: 34 up-regulated, and 2 down-regulated. All the statistically significant DE genes were concordant with the literature reports. Among the remaining 39 non-statistically significant DE genes, 9 showed an opposite direction of their expression as compared to what reported previously in literature. *AURKA* and *BIRC5*, although very promising (considering that survivin is a novel therapeutic target for lung cancer), did not show any evaluable fluorescence signal using both techniques based on Evagreen® and TaqMan® probes.

Gene Symbol	Up/Down regulation (see Appendix1)	log2(FC)	One tail-WT	BH	qvalue
TOP2A	U	3.073	**$4.84x10^{-4}$**	**$1.33x10^{-2}$**	**$6.24x10^{-3}$**
CHEK1	U	1.49	**$5.33x10^{-4}$**	**$1.33x10^{-2}$**	**$6.24x10^{-3}$**
FEN1	U	1.78	**$1.24x10^{-3}$**	**$1.86x10^{-2}$**	**$8.74x10^{-3}$**
FANCI	U	2.53	**$1.84x10^{-3}$**	**$1.97x10^{-2}$**	**$9.25x10^{-3}$**
CALB2	U	5.67	**$3.95x10^{-3}$**	**$2.96x10^{-2}$**	**$1.39x10^{-2}$**
SULF1	U	1.47	**$6.42x10^{-3}$**	**$3.79x10^{-2}$**	**$1.78x10^{-2}$**
CCNO	U	3.17	**$7.99x10^{-3}$**	**$3.79x10^{-2}$**	**$1.78x10^{-2}$**
KRT5	U	1.49	**$8.45x10^{-3}$**	**$3.79x10^{-2}$**	**$1.78x10^{-2}$**
C10orf116	D	-2.13	**$1.02x10^{-2}$**	**$4.02x10^{-2}$**	**$1.88x10^{-2}$**
SMARCA4	U	0.61	**$1.10x10^{-2}$**	**$4.02x10^{-2}$**	**$1.88x10^{-2}$**
MSLN	U	1.84	**$1.35x10^{-2}$**	**$4.60x10^{-2}$**	**$2.16x10^{-2}$**
THBS2	U	4.99	**$2.15x10^{-2}$**	$6.71x10^{-2}$	**$3.15x10^{-2}$**
AKR1C1	D	-0.22	**$2.87x10^{-2}$**	$7.69x10^{-2}$	**$3.61x10^{-2}$**
PDGFRB	U	2.79	**$2.98x10^{-2}$**	$7.69x10^{-2}$	**$3.61x10^{-2}$**
KRT18	U	0.86	**$4.63x10^{-2}$**	$9.70x10^{-2}$	**$4.55x10^{-2}$**
VEGFA	U	2.14	$8.87x10^{-2}$	$1.42x10^{-1}$	$6.63x10^{-2}$
PGK1	U	2.47	$1.12x10^{-1}$	$1.64x10^{-1}$	$7.69x10^{-2}$
CCNH	U	3.054	$1.30x10^{-1}$	$1.88x10^{-1}$	$8.80x10^{-2}$
EID1	U	0.53	$1.74x10^{-1}$	$2.37x10^{-1}$	$1.11x10^{-1}$
PECAM1	D	-0.34	$1.86x10^{-1}$	$2.50x10^{-1}$	$1.17x10^{-1}$
FAS	U	0.31	$2.06x10^{-1}$	$2.72x10^{-1}$	$1.27x10^{-1}$
PTGS2	U	0.13	$2.48x10^{-1}$	$3.21x10^{-1}$	$1.51x10^{-1}$
RHOB	U	0.28	$2.74x10^{-1}$	$3.37x10^{-1}$	$1.58x10^{-1}$
PDGFRA	*D*	*0.0027*	$2.87x10^{-1}$	$3.47x10^{-1}$	$1.63x10^{-1}$
DAP	U	0.027	$3.07x10^{-1}$	$3.66x10^{-1}$	$1.71x10^{-1}$
FGF2	U	1.61	$4.27x10^{-1}$	$5.00x10^{-1}$	$2.34x10^{-1}$
CCND2	*D*	*1.41*	$5.60x10^{-1}$	$6.46x10^{-1}$	$3.03x10^{-1}$
CDKN1A	*D*	*1.40*	$8.48x10^{-1}$	$9.08x10^{-1}$	$4.26x10^{-1}$
METAP1	*U*	*-0.36*	$8.59x10^{-1}$	$9.08x10^{-1}$	$4.26x10^{-1}$
ASPA	*U*	*-1.54*	$9.99x10^{-1}$	1	$4.69x10^{-1}$
BLMH	U	0.092	1	1	$4.69x10^{-1}$
AURKA	U	Not detected			
BIRC5	U	Not detected			

Table 14: Analyzed genes detected by both a statistically significant RTS and DM (all listed genes had a P-value of $1.77x10^{-2}$ from DM). In bold, statistically significant results at P-value<0.05. In italics, genes showing an opposite trend between what reported in literature and tissue expression verified in our study. log2 (FC)= logarithm in base 2 of Fold-Change. One tail-WT = p-value following Wilcoxon test at 1 tail. BH= p-value corrected with Benjamini & Hochberg method (1995). qvalue= p-value corrected with False Discovery Rate, (Storey, 2002).

Gene Symbol	Up/Down regulation (see Appendix1)	log2(FC)	One tail-WT	BH	qvalue
CENPF	U	1.97	**$1.82x10^{-3}$**	**$1.97x10^{-2}$**	**$9.25x10^{-2}$**
ASS1	U	2.28	**$6.44x10^{-3}$**	**$3.80x10^{-2}$**	**$1.78x10^{-2}$**
DSP	U	4.69	**$8.61x10^{-3}$**	**$3.80x10^{-2}$**	**$1.78x10^{-2}$**
ITGA4	U	1.04	**$2.57x10^{-2}$**	$7.68x10^{-2}$	**$3.60x10^{-2}$**
NME2	U	1.94	**$2.98x10^{-2}$**	$7.69x10^{-2}$	**$3.60x10^{-2}$**
SOD1	U	2.42	**$3.26x10^{-2}$**	$7.88x10^{-2}$	**$3.70x10^{-2}$**
CXADR	U	3.51	**$3.44x10^{-2}$**	$8.06x10^{-2}$	**$3.78x10^{-2}$**
PCNA	U	2.52	**$3.96x10^{-2}$**	$8.74x10^{-2}$	**$4.10x10^{-2}$**
PLK2	U	0.81	$5.69x10^{-2}$	$1.10x10^{-1}$	$5.16x10^{-2}$
BUB1B	*U*	*-0.10*	$5.72x10^{-2}$	$1.10x10^{-1}$	$5.16x10^{-2}$
EEF2	U	1.87	$6.52x10^{-2}$	$1.16x10^{-1}$	$5.46x10^{-2}$
RAD21	U	4.81	$6.68x10^{-2}$	$1.16x10^{-1}$	$5.46x10^{-2}$
MCM2	U	0.82	$9.66x10^{-2}$	$1.51x10^{-1}$	$7.07x10^{-2}$
LGALS3BP	U	1.89	$1.01x10^{-1}$	$1.52x10^{-1}$	$7.14x10^{-2}$
NR3C1	U	1.083	$2.73x10^{-1}$	$3.37x10^{-1}$	$1.56x10^{-1}$

Table 15: Analyzed genes detected by a statistically significant RTS and with a low significant level in the DM (all listed genes had a P-value of $2.03x10^{-1}$ from DM). In bold, statistically significant resulst at P-value<0.05. In italics, genes showing an opposite trend between what reported in literature and tissutal expression verified in our study. log2 (FC)= logarithm in base 2 of Fold-Change. One tail-WT = p-value following Wilcoxon test at 1 tail. BH= p-value corrected with Benjamini & Hochberg method (1995). qvalue= p-value corrected with False Discovery Rate, (Storey, 2002).

Gene Symbol	Up/Down regulation (see Appendix1)	log2(FC)	One tail-WT	BH	qvalue
COL1A1	U	4.68	**$2.76x10^{-4}$**	**$1.33x10^{-2}$**	**$6.24x10^{-3}$**
HEG1	U	1.94	**$9.02x10^{-4}$**	**$1.70x10^{-2}$**	**$7.93x10^{-3}$**
SPINT2	U	4.64	**$2.81x10^{-3}$**	**$2.64x10^{-2}$**	**$1.24x10^{-2}$**
NUSAP1	U	1.46	**$3.24x10^{-3}$**	**$2.70x10^{-2}$**	**$1.26x10^{-2}$**
MCM4	U	0.87	**$7.36x10^{-3}$**	**$3.80x10^{-2}$**	**$1.78x10^{-2}$**
TIMP3	U	1.039	**$7.80x10^{-3}$**	**$3.80x10^{-2}$**	**$1.78x10^{-2}$**
TNPO2	U	3.80	**$1.13x10^{-2}$**	**$4.02x10^{-2}$**	**$1.88x10^{-2}$**
GALNT7	U	2.28	**$1.13x10^{-2}$**	**$4.02x10^{-2}$**	**$1.88x10^{-2}$**
HCA112	U	2.89	**$2.00x10^{-2}$**	$6.52x10^{-2}$	**$3.06x10^{-2}$**
RAN	U	3.72	**$2.96x10^{-2}$**	$7.69x10^{-2}$	**$3.61x10^{-2}$**
CFB	U	1.0541	**$3.15x10^{-2}$**	$7.87x10^{-2}$	**$3.69x10^{-2}$**
UPK1B	U	5.20	**$3.95x10^{-2}$**	$8.74x10^{-2}$	**$4.10x10^{-2}$**
ALDOA	U	1.75	**$4.65x10^{-2}$**	$9.70x10^{-2}$	**$4.55x10^{-2}$**
THBS1	U	2.99	$5.60x10^{-2}$	$1.10x10^{-1}$	$5.16x10^{-2}$
SSBP1	U	2.61	$5.90x10^{-2}$	$1.11x10^{-1}$	$5.18x10^{-2}$
COL6A1	U	0.85	$6.10x10^{-2}$	$1.12x10^{-1}$	$5.23x10^{-2}$
CRIP1	U	1.45	$7.01x10^{-2}$	$1.20x10^{-1}$	$5.60x10^{-2}$
FGF9	U	2.94	$8.21x10^{-2}$	$1.36x10^{-1}$	$6.40x10^{-2}$
VCAN	U	1.48	$8.37x10^{-2}$	$1.36x10^{-1}$	$6.40x10^{-2}$
PGM1	U	0.90	$9.86x10^{-2}$	$1.51x10^{-1}$	$7.07x10^{-2}$
CDK2AP1	U	1.48	$1.57x10^{-1}$	$2.22x10^{-1}$	$1.04x10^{-1}$
RCN2	U	3.54	$1.74x10^{-1}$	$2.37x10^{-1}$	$1.11x10^{-1}$
SYNE1	U	6.51	$2.56x10^{-1}$	$3.25x10^{-1}$	$1.52x10^{-1}$
SARP1	U	1.34	$6.23x10^{-1}$	$7.07x10^{-1}$	$3.31x10^{-1}$
DAB2	U	-0.158	$6.55x10^{-1}$	$7.33x10^{-1}$	$3.44x10^{-1}$
IARS	*U*	*-0.63*	$7.53x10^{-1}$	$8.23x10^{-1}$	$3.86x10^{-1}$
ANXA4	U	-0.36	$7.57x10^{-1}$	$8.23x10^{-1}$	$3.86x10^{-1}$
IGFBP4	*D*	*1.76*	$9.66x10^{-1}$	1	$4.69x10^{-1}$
ITGA6	U	0.016	$9.85x10^{-1}$	1	$4.69x10^{-1}$

Table 16: Analyzed genes detected by (statistically significant) RTS only. In bold, statistically significant result at P-value<0.05. In italics, genes showing an opposite trend between what reported in literature and tissutal expression verified in our study. log2 (FC)= logarithm in base 2 of Fold-Change. One tail-WT = p-value following Wilcoxon test at 1 tail. BH= p-value corrected with Benjamini & Hochberg method (1995). qvalue= p-value corrected with False Discovery Rate, (Storey, 2002).

In order to validate further the results obtained in the tissues, we performed QPCR measurements of the 36 positive genes on two MPM cell lines (Mero-14 and Mero-25; MeT-5A were used as reference). The results are reported in table 17.

	Tissue samples			RTS	Mero-14 cell line			Mero-25 cell line		
	log2(FC)	**q-value**	**Up/ Down**	**Up/Down- Table S2**	**log2(FC)**	**q-value**	**Up/ Down**	**log2(FC)**	**q-value**	**Up/ Down**
AKR1C1	-0.22	**$3.61x10^{-2}$**	D	D	13.3	**$<10^{-3}$**	U	7.27	**$<10^{-3}$**	U
ALDOA	1.75	**$4.55x10^{-2}$**	U	U	-0.87	$4.01x10^{-1}$	D	0.35	$5.03x10^{-1}$	U
ASS1	2.28	**$1.78x10^{-2}$**	U	U	0.92	$1.73x10^{-1}$	U	2.44	**$<10^{-3}$**	U
CALB2	5.67	**$1.39x10^{-2}$**	U	U	-4.77	**$<10^{-3}$**	D	-0.088	$3.69x10^{-1}$	D
CCNO	3.17	**$1.78x10^{-2}$**	U	U	0.93	**$7.79x10^{-3}$**	U	1.51	**$<10^{-3}$**	U
CENPF	1.97	**$9.25x10^{-2}$**	U	U	-1.65	**$<10^{-3}$**	D	0.56	**$1.26x10^{-2}$**	U
CFB	1.054	**$3.69x10^{-2}$**	U	U	5.74	**$<10^{-3}$**	U	4.43	**$<10^{-3}$**	U
CHEK1	1.49	**$6.24x10^{-3}$**	U	U	-2.21	**$<10^{-3}$**	D	-0.0018	$6.05x10^{-1}$	D
COL1A1	4.68	**$6.24x10^{-3}$**	U	U	1.86	$4.96x10^{-1}$	U	5.50	**$<10^{-3}$**	U
CXADR	3.51	**$3.78x10^{-2}$**	U	U	0.74	$4.96x10^{-1}$	U	6.040	**$<10^{-3}$**	U
DSP	4.69	**$1.78x10^{-2}$**	U	U	0.25	$4.96x10^{-1}$	U	1.23	$5.03x10^{-1}$	U
FANCI	2.53	**$9.25x10^{-3}$**	U	U	-3.10	**$<10^{-3}$**	D	-2.42	**$<10^{-3}$**	D
FEN1	1.78	**$8.74x10^{-3}$**	U	U	-2.36	**$<10^{-3}$**	D	-0.73	$5.03x10^{-1}$	D
GALNT7	2.28	**$1.88x10^{-2}$**	U	U	5.69	**$<10^{-3}$**	U	4.78	$5.13x10^{-1}$	U
HEG1	1.94	**$7.93x10^{-3}$**	U	U	3.39	$3.09x10^{-1}$	U	3.740	$2.13x10^{-1}$	U
ITGA4	1.042	**$3.60x10^{-2}$**	U	U	3.71	**$<10^{-3}$**	U	-0.53	$4.89x10^{-1}$	D
KRT18	0.86	**$4.55x10^{-2}$**	U	U	-0.88	**$7.79x10^{-3}$**	D	0.15	$6.05x10^{-1}$	U
KRT5	1.49	**$1.78x10^{-2}$**	U	U				1.69	**$1.98x10^{-2}$**	U
MCM4	0.87	**$1.78x10^{-2}$**	U	U	0.42	$4.96x10^{-1}$	U	2.024	$5.03x10^{-1}$	U
MSLN	1.84	**$2.16x10^{-2}$**	U	U	1.086	$9.31x10^{-2}$	U	0.53	$5.69x10^{-1}$	U
NME2	1.94	**$3.60x10^{-2}$**	U	U	2.25	$6.39x10^{-1}$	U	3.48	$5.62x10^{-1}$	U
NUSAP1	1.46	**$1.26x10^{-2}$**	U	U	-2.59	**$<10^{-3}$**	D	0.016	$5.03x10^{-1}$	U
PCNA	2.52	**$4.10x10^{-2}$**	U	U	-0.020	$4.96x10^{-1}$	D	1.20	$5.03x10^{-1}$	U
PDGFRB	2.79	**$3.61x10^{-2}$**	*U*	U	9.83	**$<10^{-3}$**	*U*	8.82	**$<10^{-3}$**	*U*
RAN	3.72	**$3.61x10^{-2}$**	U	U	3.27	$9.31x10^{-2}$	U	5.025	**$4.65x10^{-2}$**	U
SMARCA4	0.61	**$1.88x10^{-2}$**	U	U	-1.70	**$<10^{-3}$**	D	-0.90	**$<10^{-3}$**	D
SOD1	2.42	**$3.70x10^{-2}$**	U	U	1.61	$6.39x10^{-1}$	U	3.93	**$5.07x10^{-2}$**	U
SULF1	1.47	**$1.78x10^{-2}$**	*U*	U	9.76	**$<10^{-3}$**	*U*	14.19	**$<10^{-3}$**	*U*
THBS2	4.99	**$3.15x10^{-2}$**	*U*	U	9.65	**$<10^{-3}$**	*U*	7.70	**$<10^{-3}$**	*U*
TIMP3	1.039	**$1.78x10^{-2}$**	*U*	U	0.98	**$7.79x10^{-3}$**	*U*	4.18	**$<10^{-3}$**	*U*
TNPO2	3.80	**$1.88x10^{-2}$**	U	U	2.94	$6.39x10^{-1}$	U	1.017	$5.03x10^{-1}$	U
TOP2A	3.073	**$6.24x10^{-3}$**	U	U	-1.98	**$<10^{-3}$**	D	-0.92	**$<10^{-3}$**	D

Table 17: Comparison between results from RTS, measurements in tissue samples, and measurements performed in Mero-14 and Mero-25 cell lines. Only genes positive in tissues were measured in cell lines. In bold, statistically significant result at P-value<0.05. In italics, genes showing an opposite trend between what reported in literature and tissutal expression verified in our study. log2 (FC)= logarithm in base 2 of Fold-Change. qvalue= p-value corrected with False Discovery Rate, (Storey, 2002).

Comparing the results obtained on the tissues with those on cell lines, it should be noticed that:

• 6 genes (*CCNO*, *CFB*, *PDGFRB*, *SULF1*, *THBS2*, *TIMP3*) confirmed the same direction (all up-regulated) in tissues and both cell lines, in a statistically significant way;

• 2 genes (*GALNT7* and *ITGA4*) confirmed the same direction (both up-regulated) in tissues and in Mero-14 cell line in a statistically significant way (*GALNT7* shows also the same direction in Mero-25, although in a not-statistically significant way);

• 6 genes (*ASS1*, *CENPF*, *COL1A1*, *CXADR*, *KRT5*, and *RAN*) showed the same trend (all up-regulated) in tissues and in Mero-25 cell line in a statistically significant way (*COL1A1* and *RAN* showed the same direction also in Mero-14, although in a not-statistically significant way);

• 4 genes (*AKR1C1*, *FANCI*, *SMARCA4*, and *TOP2A*) showed the same trend both in Mero-14 and Mero-25, but they were in the opposite direction as compared to the tissues;

The remaining 14 genes were found up-regulated in a statistically significant way only in tissues. However, among these genes, *HEG1*, *NME2*, and *SOD1* showed the same direction also in both the cell lines. Moreover, *TNPO2*, *PCNA*, and *MCM4* showed the same trend in Mero-25, while *MSLN* showed a trend of up-regulation in Mero-14 cell line, close to the statistical significance.

In summary, *ASS1*, *CCNO*, *CENPF*, *CFB*, *COL1A1*, *CXADR*, *GALNT7*, *ITGA4*, *KRT5*, *PDGFRB*, *RAN*, *SULF1*, *THBS2*, and *TIMP3* were genes deregulated in tissues confirmed in at least one cell line. Moreover, at least one cell line showed the same trend found in tissues (close to a statistical significance) for *MSLN*, *TNPO2*, *NME2*, *HEG1*, *PCNA*, *SOD1*, and *MCM4*.

4.3 Mutation screening of *PDGFRB*

Among the analyzed genes, an interesting result is the up-regulation of *PDGFRB* (NM_002609). It encodes for a tyrosine-kinase receptor, member of the platelet-derived growth factor (PDGF) family. PDGF is composed of homo-dimers or hetero-dimers of two polypeptide chains, denoted A and B. These polypeptides are encoded by two distinct genes, showing a high degree of similarity (Betsholtz et al., 1986). All three dimeric combinations (AA, AB and BB) were identified (Stroobant and Waterfield, 1984; Heldin et al., 1986; Hammacher et al., 1986). Based on ligand-binding and cross-competition analysis, two different PDGF receptors, alpha and beta, have been described (Hart et al., 1988; Heldin et al., 1986). The PDGF-alpha receptor binds all three forms with high affinity, whereas the beta-receptor subtype only binds PDGF-BB with high affinity (Claesson-Welsh et al., 1988; Hammacher et al., 1988; Seifert et al., 1989). Early, it was observed that a panel of human MPM cell lines express PDGF beta-chain and PDGF beta-receptor transcripts, whereas normal mesothelial cell lines express no PDGF B-chain mRNA and little or no PDGF beta-receptor mRNA (Gerwin et al., 1987; Versnel et al., 1991). It has been suggested that normal mesothelium may be responsive to PDGF by the predominant expression of PDGFR-alpha, whereas MPM is subjected to a growth self-stimulation loop through PDGF/PDGFR-beta interaction (Ascoli et al., 1995). More recent experiments seemed to elucidate a different mechanism of tumorigenesis, not involving the autocrine stimulation via PDGF, rather suggesting a mechanism of paracrine growth stimulation in MPM (Metheny-Barlow et al., 2001). However, all these findings indicated that *PDGFRB* could be an interesting therapeutic target for MPM, at least for those patients expressing *PDGFRB* in the tumour cells. Following this hypothesis, tyrosine kinase inhibitors were assayed as potential new drugs against MPM. One of the most promising drug for targeted therapy is Imatinib (imatinib mesylate, STI571; Gleevec or Glivec). Imatinib is an ATP-competitive inhibitor that has revolutionized drug therapy of chronic myeloid leukemia. It induced cytotoxicity and apoptosis selectively on *PDGFRB* positive MPM cells via blockade of receptor phosphorylation and interference with the Akt pathway (Druker, 2006). Personal communication (reported by Dr. L. Mutti, Hospital of Vercelli, Italy) highlighted that *PDGFRB* was assayed for compassionate use in advanced MPM patients in a pre-clinical trial where imatinib was administered with gemcitabine, in a combined targeted therapy protocol. Preliminary observations reported that patients showed a good response with the stabilization or partial shrinkage of the tumor mass. However, some patients either did not respond or, after an initial response, relapsed.

In other tumour types, the causes for the resistance to imatinib were, at least in part, ascertained. Some specific mutations, the gatekeeper mutations (like T670 and T681) within *PDGFRB*, make cells insensitive to imatinib. Therefore, gastro-intestinal stromal tumor (GIST) patients with these mutations respond poorly to imatinib (Corless et al., 2005; Heinrich et al., 2006). The resistance to imatinib usually results from the emergence of tumour clones with mutations that prevent drug binding (Blencke et al., 2004; Böhmer et al., 2003; Heinrich et al., 2006). The question arises whether the mechanisms of lack of response to imatinib in MPM patients are similar to those observed in GIST. The frequency of mutations within *PDGFRB* at the presentation in MPM is unknown. Moreover, it is also unknown whether in the MPM the resistance to Imatinib could be ascribed to specific mutations within *PDGFRB*, similarly to what observed in GlST.

To measure the frequency of these events, we performed a mutation screening of exons 12 and 18 of *PDGFRB*, the two exons that commonly show activating somatic mutations in human cancers (e.g. in the GIST), in 100 surgically resected MPMs, and we did not found any mutation, as reported in table 18.

Number of MPM	Exon 12	Exon 18
92	wt	wt
4	NA	NA
2	NA	wt
1	wt	NA
1	wt	c.2523G>A p.K841K

Table 18: Results of the mutation screening of *PDGFRB* (wt: wild type; NA: no-amplification).

This led to conclude that the malignancy is not driven by somatic mutations of this gene, but an increased expression could be functionally equivalent to a constitutive activation of the receptor. Thus, imatinib could be helpful in patients where *PDGFRB* is over-expressed. In any case, the lack of response to imatinib should not be ascribed to mutant *PDGFRB*, at the presentation. Concerning the relapse, we studied an *in vitro* model. To ascertain whether *PDGFRB* plays a role in the secondary resistance to imatinib, we induced a long-term resistance to imatinib in the over-expressing *PDGFRB* Mero-14 cells, and we screened the resistant clones for mutations in exons 12, 14, and 18 by using Next Generation Sequencing. Again, we could not detect any mutation. Moreover, resistant cells were sequenced for *c-KIT*, and *c-MET*, two tyrosine kinase receptors, frequently mutated in neoplastic diseases, without finding any mutation

4.4 Functional study on Mesothelin and Calretinin

4.4.1 Phenotypic tests after knockdown of target genes

Out of the 77 genes analyzed, also the high levels of *MSLN*, and *CALB2* in MPM samples captured our interest, given that these genes are both biomarkers useful for diagnosis and prognosis. Unfortunately, few is known about their biological functions. For this reason, in order to ascertain whether these genes play some role in maintaining the malignant phenotype, rather to be simply epiphenomenons, we used a gene silencing-RNA approach (this part of the work was carried out at the laboratories of Prof. Justin Stebbing, and Dr. Georgious Giamas at the Imperial College of London, UK).

The first step was to verify the expression of *MSLN* and *CALB2* in MPM cell lines at protein level. Thus, we screened MeT-5A (as reference), and Mero-14 and Mero-25 MPM cell lines for MSLN and CALB2 with western blot. We found that Mero-14 over-expressed MSLN as compared to MeT-5A and Mero-25: the difference appeared proportionally more relevant in comparison to the increase of mRNA. Mero-14 and Mero-25 were also not expressing CALB2, thus we screened more cell lines. Thanks to the collaboration with Antonio Daga (Istituto Tumori, Genova) we could obtain one cell line (IstMes2), over-expressing CALB2 at protein level. Moreover, in order to perform more experiments on mesothelin, we included also the cell line NCI-H28, one MPM cell line not expressing MSLN. Thus, for all these cell lines we repeated the measurements both of the mRNA and the protein levels and the results, confirming our expectations, are reported in figure 13.

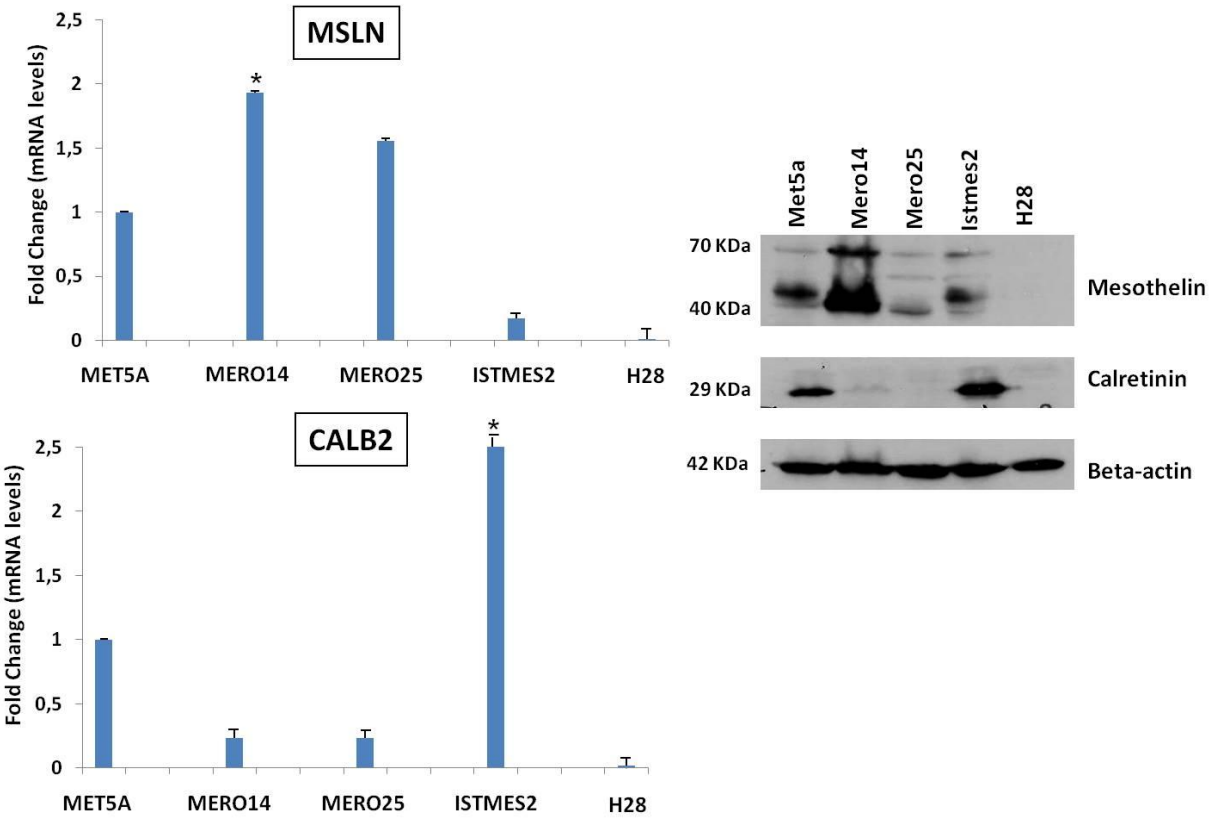

Fig. 13: Basal expression level of MSLN and CALB2 in four MPM cell lines. On the left, RTqPCR showing fold change of quantitative mRNA levels of *MSLN* (on the top), and *CALB2* (on the bottom) measured on MPM cell lines versus MeT-5A cells. Mean of *RPLP0*, *HPRT*, and *TBP* was used for normalization. Errors bars are SEM of the fold change from three independent experiments, each in triplicate. Mero-14 cells showed the highest expression levels of *MSLN* ($p = 0.02$). IstMes2 cells showed the highest expression level of *CALB2* ($p = 0.017$). On the right, the protein levels of MSLN and CALB2 on MeT-5A, Mero-14, Mero 25, IstMes2, and NCI-H28 cells. β-Actin was used as loading control. The protein levels were confirmed with two independent experiments.

We tested two different siRNAs to get an optimal depletion of *MSLN*, and other 2 for the depletion of *CALB2*. The knockdown of *MSLN* was about 90% with both the siRNAs, so we have arbitrarily chosen the *MSLN* siRNA 1 at a concentration of 40 nM. Regarding *CALB2*, even if at mRNA level, the siRNA 6 shows to be more effective, at protein level siRNA 5 seemed more efficient. For this reason we have chosen the latter siRNA, at a concentration of 50 nM (Figure 14). However, it should be observed that the silencing of *CALB2* was not optimal (it was observed only a reduction of about 50% of the protein), and not better results could be obtained.

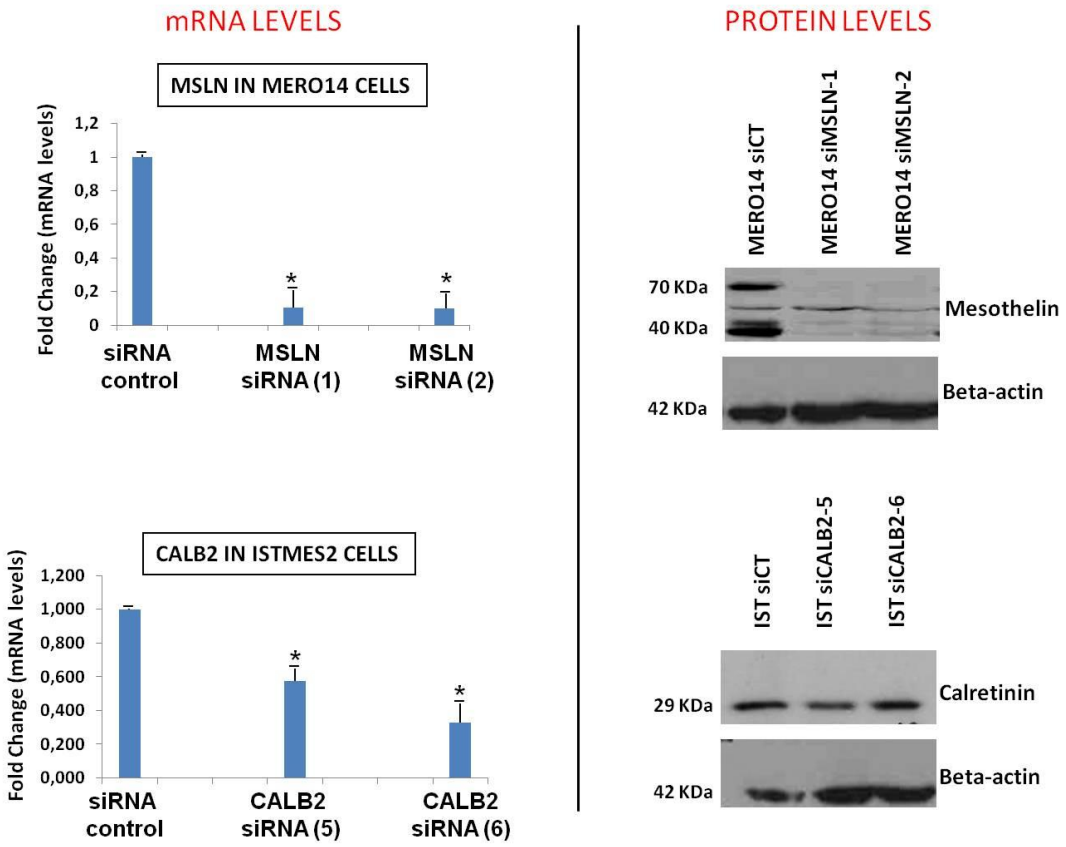

Fig. 14: Silencing of MSLN and CALB2 genes. On the left, RTqPCR showing fold change of quantitative mRNA levels of *MSLN* in Mero-14 cells (on the top), and *CALB2* in IstMes2 cells (on the bottom) measured to detect the best siRNA to be used. Mean of *RPLP0*, *HPRT*, and *TBP* was used for normalization. Errors bars are SEM of the fold change from three independent experiments, each in triplicate. The siRNAs chosen for the analysis are *MSLN* siRNA 1 (40 nmol; *P=0.002) on Mero-14 cells, and *CALB2* siRNA 5 (50 nmol; *P =0.013) on IstMes2 cells. On the right, the protein levels of *MSLN* after its depletion with *MSLN* siRNA 1 and 2 (40 nmol) versus the control, and of *CALB2* after its depletion with *CALB2* siRNA 5 and 6 (50 nmol) versus the control. β-Actin was used as loading control. The protein levels were confirmed with three independent experiments.

After having confirmed the depletion of the target genes in the relevant cell lines, we studied the behavior of Mero-14 and IstMes2 cell lines for their culture growth parameters, cell cycle, migration, invasion, and apoptosis.

siRNA *and Growth*

As shown in fig. 15, we observed a significant reduction ($p < 0.05$) in the proliferation rate of Mero-14 cells starting from the third day of treatment with *MSLN* siRNA, and increasing until the 6th day, end of the experimental procedure. To elucidate the molecular mechanism underlying this phenotypic effect, we evaluated the expression of some of the most important proteins involved in the signaling pathways of metastasis and cell proliferation, as pAKT and pERK, and we have noticed that following *MSLN* silencing, we get a reduction of protein expression levels of pAKT and pERK. As further step, we induced a transient over-expression of *MSLN* in the NCI-H28 cells that do not express endogen levels of *MSLN* at RNA and protein levels. Reassuringly, we measured increased levels of pAKT and pERK, confirming the link between *MSLN* expression, proliferation and phosphorylation status of AKT and ERK.

Then, we tested the proliferation rate of IstMes2 cells following the *CALB2* knockdown, but we did not observe any change for this parameter.

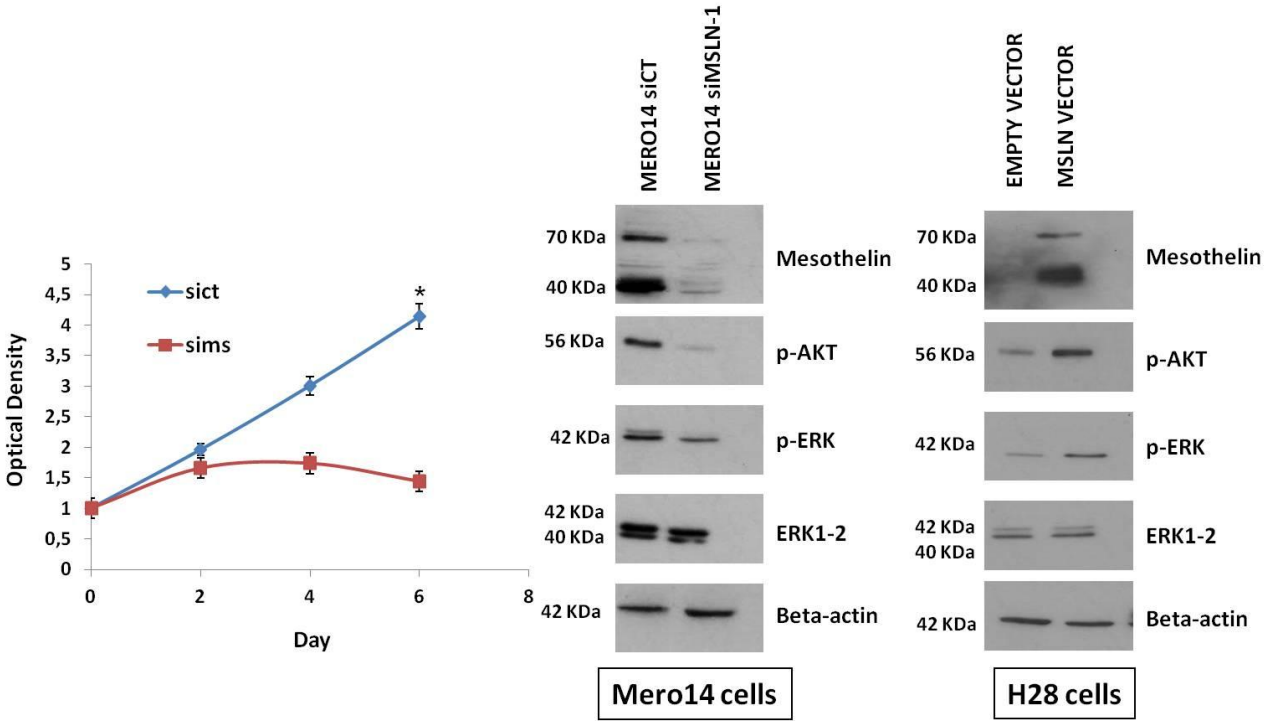

Fig. 15: Effect of MSLN on the proliferation. On the left, proliferation assay in Mero-14 cells treated with 40 nmol of the Control siRNA (siCt), and *MSLN* siRNA (siMsln). *P = 0.04. Error bars represent SEM of three separate experiments, each done in quadruplicate. On the right, Western blotting analysis of Mero-14-control, Mero-14-*MSLN* siRNA, NCI-H28-empty vector, and NCI-H28-overexpressing *MSLN* vector with indicated antibodies. β-Actin was used as loading control. The protein levels were confirmed with three independent experiments.

We studied further the growth by employing the 3D Matrigel-overlay model, where the conditions have more similarities with the physiological growth conditions, allowing the integration of crucial extracellular matrix signaling. In the tridimensional contest, after having silenced *MSLN*, Mero-14 cells formed spheres smaller and more uniform, acquiring a shape closely resembling that of nonmalignant cells, compared to the control. With the depletion of *CALB2*, the spheres were bigger and they lost morphological uniformity. The cells formed grape-like complexes, characterized by the absence of functional cell-cell adhesion and increased invasive behavior (Fig. 16). We have also measured the dimension of the single spheres created by Mero-14 cells. We got 72.5% of the spheres bigger than 40 µm in the control group, and 22.5% in the group treated with *MSLN* siRNA. The measure of grape-like structures formed by IstMes2, and the consequential comparing with the control group were not possible because of the characteristic unusual jagged shape obtained following the depletion of *CALB2*.

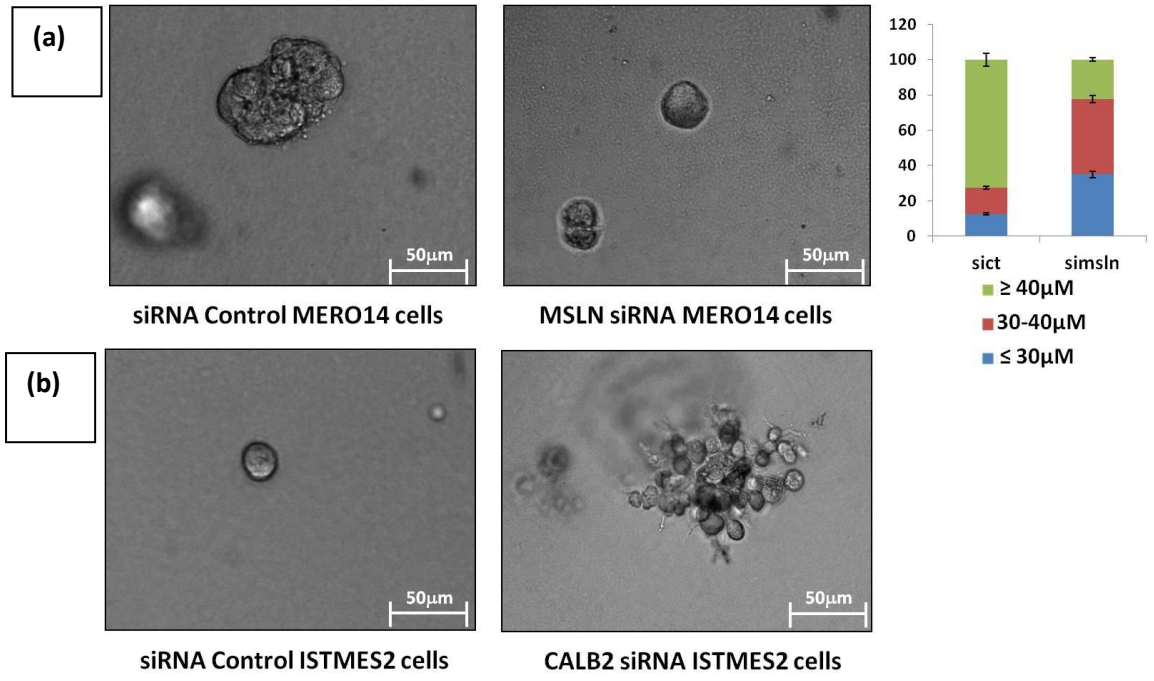

Fig. 16: Effect of gene silencing on the growth on 3D-matrigel. *MSLN* and *CALB2* knock-down affect morphological features of Mero-14 and IstMes2 cell lines respectively. The picture represents the phase contrast microscopy of Mero-14 (a), and IstMes2 (b) cells cultured in 3D Matrigel-overlay chambers after silencing of *MSLN* 40nM (a), and *CALB2* 50nM (b). Magnification 10X. Two different experiments, each in triplicate. On the right, the percentage of Mero-14 cells, having different dimension in the group treated with *MSLN* siRNA, versus the control.

siRNA *and Cell cycle*

To examine the effect of *MSLN* and *CALB2* silencing on cell cycle progression, Mero-14 and IstMes2 cells were treated with CT siRNA or *MSLN* and *CALB2* siRNA for 72 h and analyzed with FACS Diva Software, allowing measuring the share of cells in each stage of the cell cycle.

We observed a decreased share of cells in S/G_2M-phases following the treatment with the *MSLN* siRNA (although the difference with controls was not statistically significant) (Figure 17). No significant differences were noticed after depletion of *CALB2* (not showed).

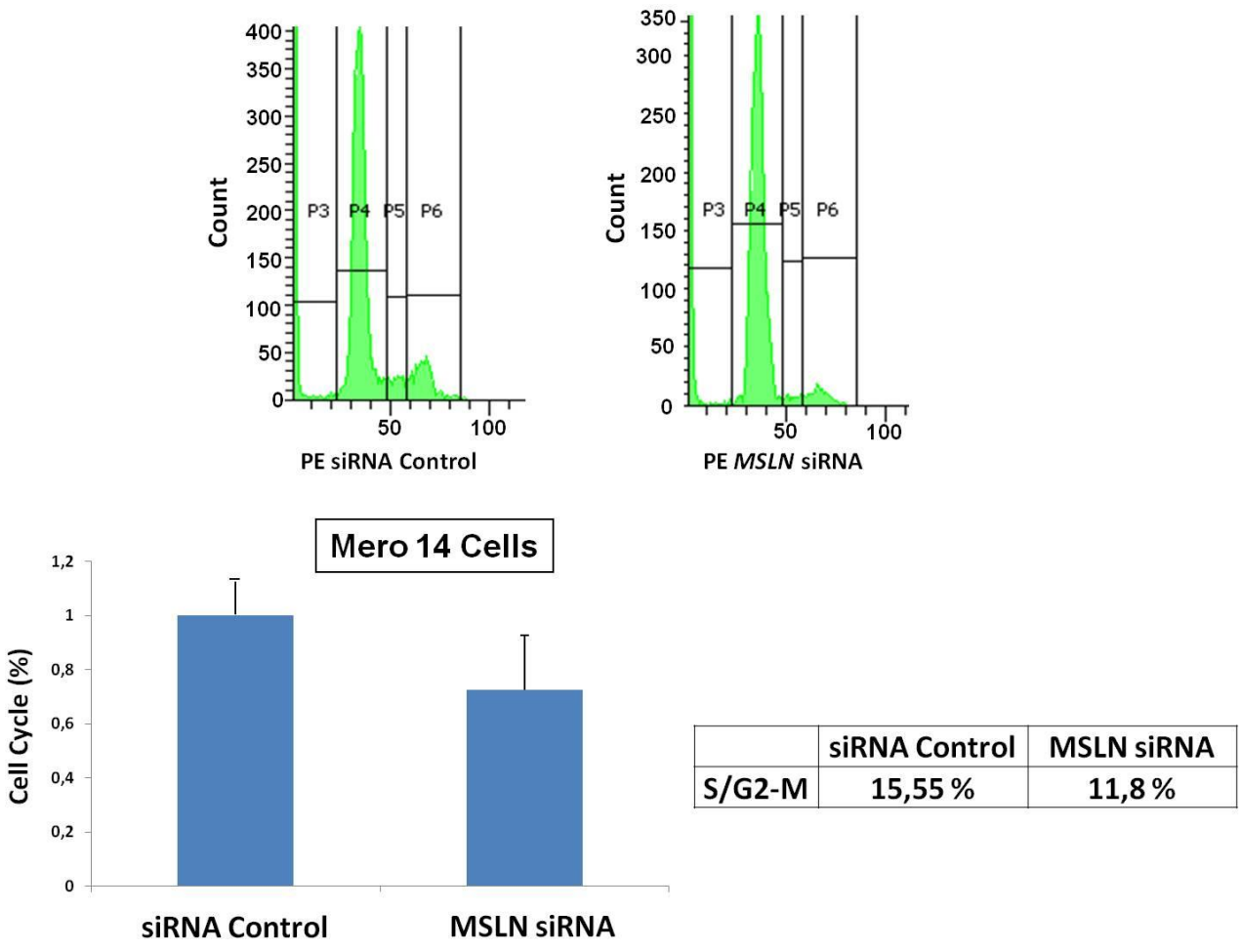

Fig. 17: Progression of cell cycle. The graph shows the percentage of cells in phase S/G_2M in Mero-14 treated with with 40 nmol of the Control siRNA, and *MSLN* siRNA. Error bars represent SEM of four separate experiments.

siRNA *and Migration/Invasion*

With the Wound-Healing Assay we have monitored the migration of cells into a scratch made in a confluent monolayer, after the knockdown of *MSLN* and *CALB2* within 72h. We could not detect any statistically significant difference for both the treatments (figure 18). On the contrary, the invasion, performed with the trans-well assay, showed a reduction (indicative of a reduced metastatic capability) of the Mero-14 cells ($p < 0.05$), after 48h of treatment with *MSLN* siRNA. On the other hand, even if in not statistical significant way, the depletion of *CALB2* increased invasiveness, according with the findings obtained with the 3D Matrigel-overlay model (Fig. 19).

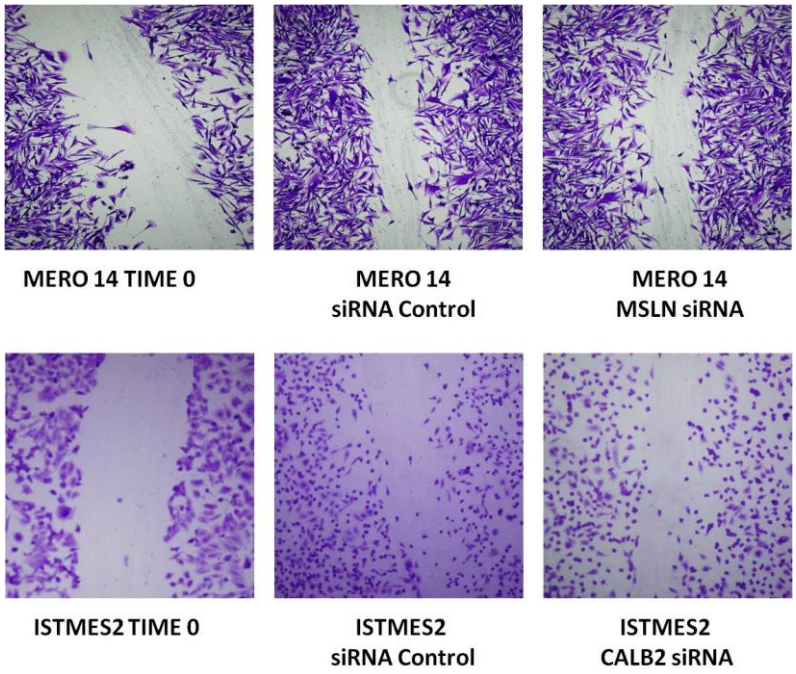

Fig. 18: Scratch assay of gene silenced cells. Confluent monolayers of Mero-14 cells on the top, and IstMes2 cells on the bottom, were pretreated for 72 hr with 40 nmol of the Control siRNA, and *MSLN* siRNA, and 50 nmol of the Control siRNA, and *CALB2* siRNA respectively. After introducing a scratch, cells were incubated in full medium and visualized 36 hr later the Mero-14 cells, and 20 hr later the IstMes2 cells. Two different experiments were performed, each in triplicate.

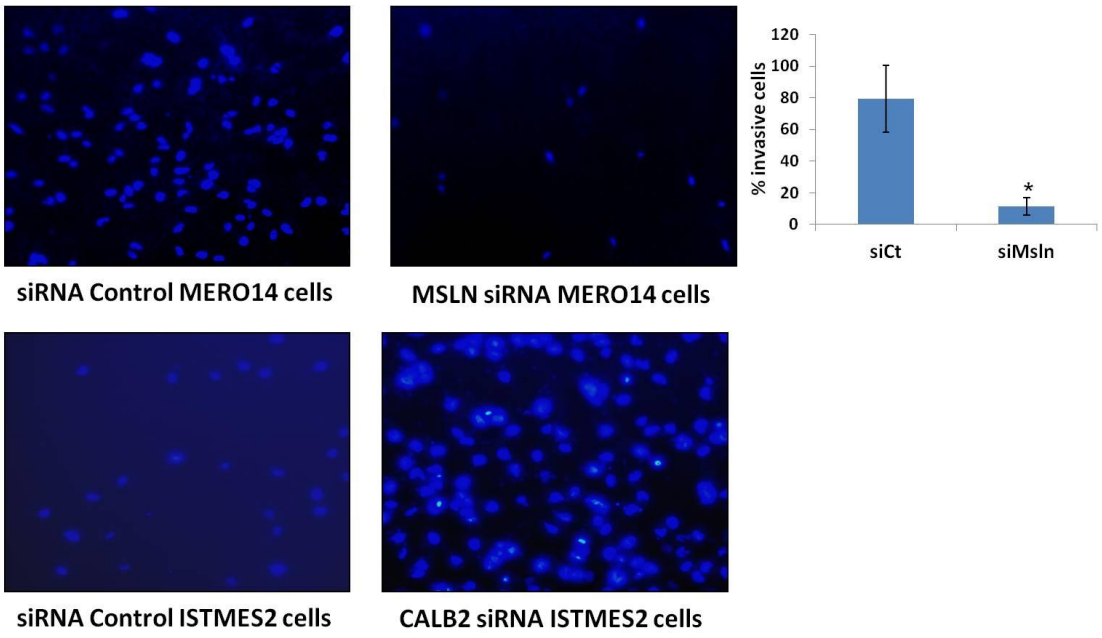

Fig. 19: Transwell assay of gene silenced cells. On the top, transwell invasion assay of Mero-14 cells treated with 40 nmol of the Control siRNA, and *MSLN* siRNA (*P = 0.0044). Error bars represent SEM of two separate experiments, each done in triplicate. On the bottom, the picture of the transwell invasion assay of IstMes2 cells treated with 50 nmol of the Control siRNA, and *CALB2* siRNA.

siRNA *and Apoptosis*

Subsequently, we measured the apoptotic level of MPM cells after the knockdown of *MSLN* and *CALB2*. We observed that the depletion of the target genes did not affect the degree of caspases released (data not showed).

4.4.2 Combination between MSLN/CALB2 siRNA *and drugs*

We tested the effect of the gene silencing (either *MSLN* or *CALB2*), and the administration of chemotherapeutics drugs including cisplatin, commonly used in MPM patients, gemcitabine, and imatinib, promising drugs next to be assayed in clinical trials, alone, or in various combinations. Following these treatments, we studied the proliferation rates, the cell cycle, and the apoptosis of Mero-14 and IstMes2.

siRNA+*chemotherapeutic drugs and proliferation*

Regarding the proliferation assay, we observed an additive effect when Mero-14 cells were treated with *MSLN* siRNA alone, cisplatin 5 µM alone, or the combination between the two agents (Figure 20) ($p < 0.05$). For *CALB2* siRNA on IstMes2, or the use of gemcitabine/imatinib (alone or in combination with or without siRNAs, in both the cell lines) we did not observe any effect and, for brevity, data were not showed.

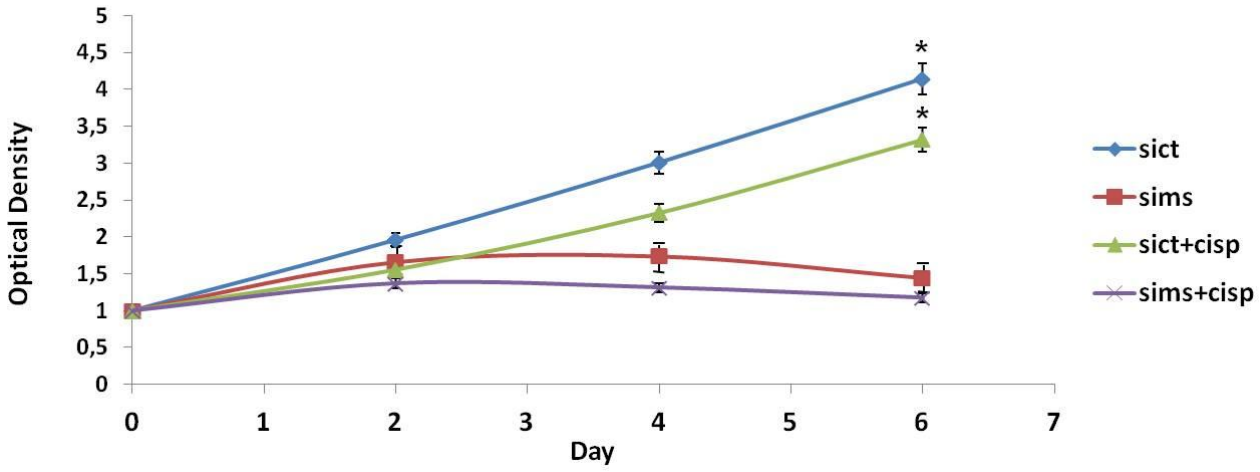

Fig. 20: Proliferation assay in Mero-14 cells treated with 40 nmol of the Control siRNA (siCt), plus 5 µM of Cisplatin, and *MSLN* siRNA, plus 5 µM of Cisplatin. The graph shows an additive effect between *MSLN* siRNA, and 5 µM of Cisplatin, compared with the single reagents (*P=0.009). Error bars represent SEM of three separate experiments, each done in quadruplicate.

siRNA+ *chemotherapeutic drugs and Cell cycle*

We did not observe any significant difference on the cell cycle progression after the treatment with *MSLN*/*CALB2* siRNA and different concentrations (5-10-20 µM) of cisplatin, gemcitabine, imatinib, and the combination between gemcitabine, and imatinib. However, a slight reduction of the share of cells in the S+G_2+M phase of the cell cycle was also observed in *MSLN* siRNA treated cells, as compared to Control-siRNA, in presence of cisplatin, confirming the same trend found in previous experiments when cisplatin was not added to the cultures.

Intriguingly, combining the experiments (those with and without cisplatin), a statistically significant difference could be observed where the share of cells in the S+G_2+M phase of the cell cycle was slightly reduced following the treatment with *MSLN* siRNA (Fig. 21).

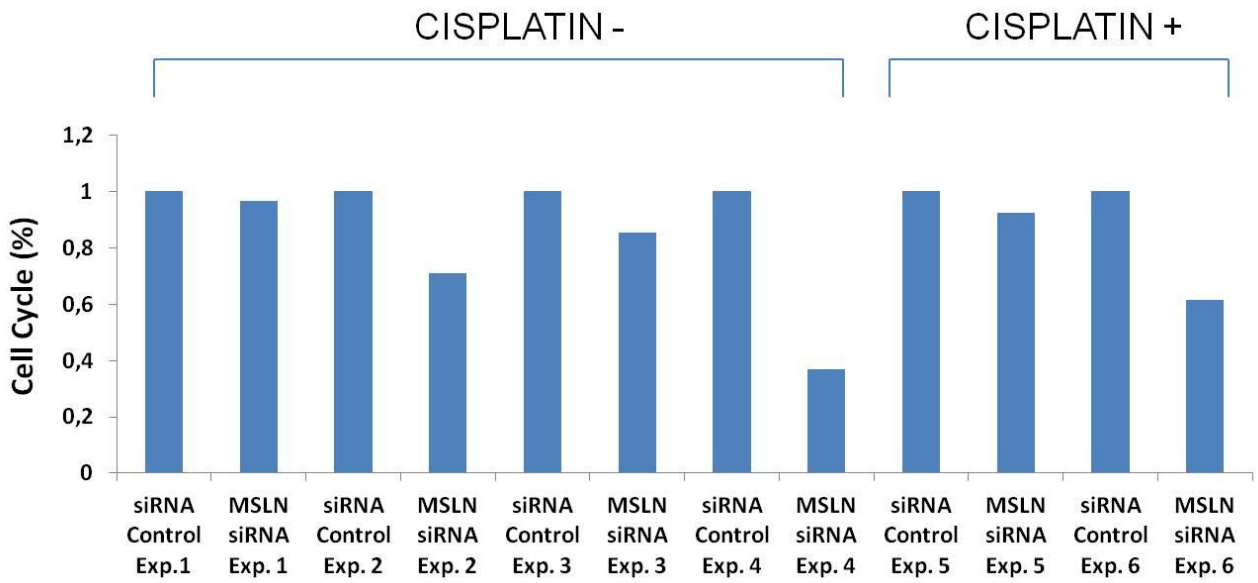

Fig. 21: Progression of cell cycle. The graph shows the percentage of cells in phase S/G_2M in Mero-14 treated with 40 nmol of the Control siRNA, and *MSLN* siRNA for the individual experiments (Exp.1–Exp.6) with and without cisplatin 5 µM. The S+G_2+M phase of the cell cycle was slightly reduced following the treatment with MSLN-siRNA (*P= 0.022).

siRNA+*chemotherapeutic drugs and apoptosis*

The combined effect detected between *MSLN* siRNA and cisplatin 5 µM in the proliferation assay could be explained by results obtained when apoptosis was measured in Mero-14 cells. The *Caspase - Glo® 3/7* assay employed measures the amount of caspase 3 and 7, involved in the intrinsic apoptotic pathway, where high absorbance values correspond to a high amount of caspases released (i.e. increased apoptosis). Interestingly, we found a synergistic effect of the treatment with *MSLN* siRNA and cisplatin. *MSLN* siRNA or cisplatin alone, do not seem to be able to induce apoptosis on Mero-14 cells. However, combined together, the cells show a statistically significant increased apoptosis, as it can be seen in Figure 22. In order to further investigate this finding, we tested some of the most important proteins involved in the apoptotic process, such as p53, and PARP. As shown in Figure 22, the combination between *MSLN* siRNA, and cisplatin 5-10-20 µM led to a stronger expression of the pro-apoptotic signals (i.e. up-regulation of p53 and PARP cleavage), compared with the treatments performed with *MSLN* siRNA or cisplatin alone. The effect on apoptosis is visible already from 5 µM of cisplatin, and it is more evident when 10µM is used.

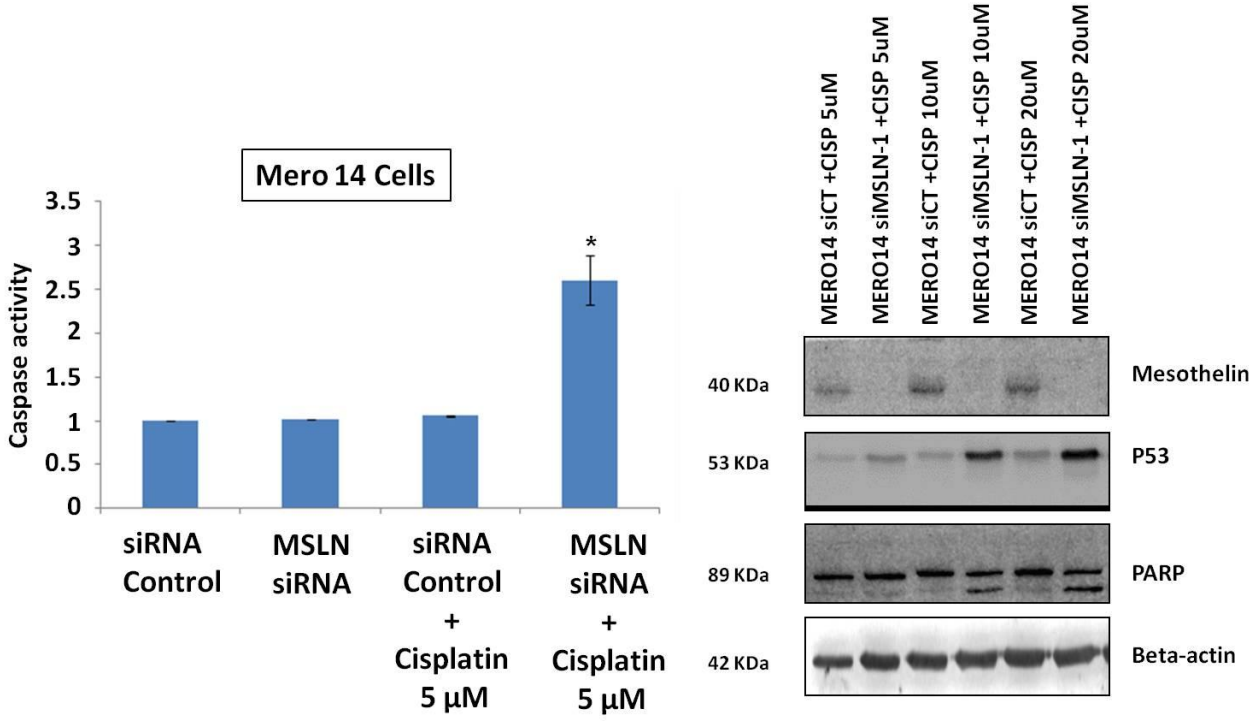

Fig. 22: Interaction between cisplatin and MSLN-siRNA **on the apoptosis of Mero-14 cells.** On the left, apoptosis assay performed on Mero-14 cells treated with 40 nmol of the Control siRNA, plus 5 µM of Cisplatin, and *MSLN* siRNA, plus 5 µM of Cisplatin. The graph shows a synergistic effect between *MSLN* siRNA, and 5 µM of Cisplatin, compared with the single reagents (*P=0,018). Error bars represent SEM of three independent experiments, each done in triplicate. On the right, Western blotting analysis of Mero-14-control plus 5 µM of Cisplatin, Mero-14-*MSLN* siRNA plus 5 µM of Cisplatin, Mero-14-control plus 10 µM of Cisplatin, Mero-14-*MSLN* siRNA plus 10 µM of Cisplatin, and Mero-14-control plus 20 µM of Cisplatin, Mero-14-*MSLN* siRNA plus 20 µM of Cisplatin, with indicated antibodies. β-Actin was used as loading control. The protein levels were confirmed with three independent experiments.

5. Discussion

The high lethality of MPM results from the aggressive phenotype, the difficulty of an early diagnosis, and the inability of current treatments to cure the disease. MPM is a tumor poorly studied (mainly due to its low incidence), and there is the need to identify the role of genes/pathways in the development of the MPM and to obtain new therapeutic targets for a development of a specific targeted therapy.

In the present study, starting from a huge panel of genes analyzed through microarrays in different publications, we focused our attention on a group of RNA markers that more redundantly appeared as de-regulated in the MPM, and that could be potentially involved in the carcinogenesis of this neoplasm. The screening we made could help to identify a set of genes useful for diagnosis and, potentially, for personalized therapies. Indeed, it is well known that every tumor is extremely heterogeneous, and it is likely that, in addition to the distinction between the known subtypes of MPM, differences between individuals with the same histotype of tumor are present.

We found that the deregulated genes are mainly involved in processes as DNA replication, and cell-cell, and cell-matrix interaction. Genes such as *FEN1*, *MCM4*, *CCNO*, *AKR1C1* and *PCNA* are markers of proliferation, and their over-expression at the RNA level is in agreement with other experimental evidence. Indeed, *FEN1* was found up-regulated in metastatic prostate cancer cells (Latulippe et al., 2002), gastric cancer cells (Kim et al., 2005), neuroblastomas (Krause et al. 2005) and in lung cancer cell lines (Sato et al., 2003). The elevated expression of *MCM4* in lung cancer, compared to the adjacent bronchial epithelial cells or various normal tissues, is consistent with the fact that MCM proteins are highly expressed in cancer cells and in human pre-cancerous cells, while they are not expressed in differentiated somatic cells and in quiescent cells (Freeman et al., 1999). *AKR1C1*, a member of the aldo-keto reductase superfamily, is associated with decreased levels of progesterone, and it is involved in proliferation, and differentiation of endometrial cells. Inhibitors of *AKR1C1* have been suggested as potential agents for treatment of endometrial cancer and endometriosis (Brozic et al., 2009). In some human cancers, such as gastric cancer, an increased immunoreactivity of *PCNA* has been related to a worse prognosis (Jain et al., 1991). Concerning the interaction cell-cell and cell-matrix, among the genes significantly up-regulated in our cases of MPM, we can find components of the cellular matrix (as *COL1A1* and *TIMP3*), cell adhesion molecules (CAM integrins, as *ITGA4*), receptors of growth factors, and glycoproteins (as *THBS2*, and *MSLN*). Cell adhesion allows and promotes biological processes such as growth, survival, migration and metastasis. In addition, it is one of the causes

for the drug resistance in blood cancers as well as in neuroblastoma, MPM, pancreatic, lung and breast cancer. Finally, it protects against cell death due to radiation, genotoxic chemotherapy, or targeted inhibitors (Schmidmaier and Baumann, 2008). Worthy of note is also the over-expression of *TOP2A*, and *SULF1*: *TOP2A* encodes a DNA topoisomerase, proposed as a negative prognostic marker of the breast cancer and it has also been associated with a positive response to anthracycline-based therapy (Brase et al., 2010). Roe et al., (Roe et al., 2010) were the first to detect the over-expression of *TOP2A* in cases of MPM, confirmed by our work. The protein encoded by the gene *SULF1* modulates the activity of many growth factors and signaling molecules. This gene is clearly over-expressed in brain cancer, breast cancer, colon adenocarcinoma, skin carcinoma, esophageal and gastric cancer, tumors of the head and neck, lung cancer, MPM, pancreatic cancer, and sarcoma (Rosen et al., 2010).

However, out of the analyzed genes, the high levels of *MSLN*, and *CALB2* in the MPM group captured our interest, appearing as the most promising MGs from which to start functional studies, by using RNA interference, and pharmacological approach.

The properties of calretinin and its role in carcinogenesis of the MPM are not known, but experimental evidences (Kinoshita et al., 2013; Kao et al., 2011) show that this marker is up-regulated in malignant pleural tissues, with immuno-histochemical analysis, and that, at diagnostics level, it is a marker commonly employed for discriminating the MPM especially of epithelial type, from other forms of cancer, such as the lung adenocarcinoma (Hyun et al., 2012; Mohammad et al., 2012). Up to now, only a scientific paper reported the increase of mRNA expression of *CALB2* (Melotti et al., 2010). In the colorectal cancer cells (CRC), Stevenson et al., have investigated the role of *CALB2*, in regulating the response to 5-Fluorouracil (5-FU). They have reported that 5-FU-induced apoptosis was significantly reduced in CRC cell lines in which *CALB2* expression had been silenced, suggesting that following 5-FU treatment in CRC cell lines, *CALB2* is involved in apoptosis induction through the intrinsic mitochondrial pathway. Moreover, down-regulation of *CALB2* in response to 5-FU may represent an intrinsic mechanism of resistance to this anti-cancer drugs (Stevenson et al., 2011). Gander et al., have observed that after the depletion of *CALB2* in the colon adenocarcinoma cell line WiDr, the morphology of these epithelial cells changed from a polygonal to a spherical shape and they formed dense cell clusters, displaying morphological alterations typical for apoptotic cells. Moreover, they have reported also an accumulation of cells in phase G_1, highlighting that *CALB2* may have an impact on the progression of the cell cycle of WiDr cells (Gander et al., 1996). On the contrary, the silencing of *CALB2* did not produce significant effects in our experimental system. The only effect obtained

did not promote tumor regression, but increased the motility and the invasion capabilities of cancer cells, indicating a probable involvement of this protein in a feed-back mechanism. An observation worthy of note, in the IstMes2 cells, and that could explain the lack of positive phenotypic effects, following *CALB2* depletion is that the decrease in the levels of the target gene was not optimal, despite many trials setting, made by varying the concentrations of siRNA, times of treatment, and types of siRNA. The decrease of calretinin that we obtained was in fact around 50%, perhaps not sufficient to get evidences on the malignant phenotype. It is plausible indeed, that the 50% of calretinin, still active was sufficient for the cells to maintain their characteristic picture of malignancy. On the other hand, however, it should be noticed that the other MPM cell lines, that we have previously screened for *CALB2*, did not express high levels of this target. So, it is not clear whether the results we have achieved depend on the extreme variability, and heterogeneity of MPM, or by the fact that the calretinin is not a gene closely linked to tumorigenesis, but only an epiphenomenon.

The present study highlighted the important role of *MSLN* in the carcinogenesis of the MPM. Indeed, the *MSLN* transient genetic silencing in Mero-14 cells compromised their proliferation rate, their invasion capacity, and their ability to form big spheroids. From this experimental evidences, it seems that the depletion of *MSLN* causes the arrest of some of the most important characteristics malignant phenotypes. For this reason, *MSLN* could be considered not only a promising diagnostic marker, but also a molecular target for the cure of the MPM. With this work we have confirmed findings already revealed by Wang et al. They have reported that specific gene silencing for *MSLN* by distinct methods (siRNA and microRNA) decreased viability of cancer cells from different origins, such as MPM (H2373), ovarian cancer (Skov3 and Ovcar-5) and pancreatic cancer (Miapaca2 and Panc-1). Additionally, the invasiveness of cancer cells was also significantly decreased upon such treatment, suggesting that the molecular mechanism of reduced invasiveness was connected to the reduced expression of b-Catenin, an important marker of EMT (epithelial-mesenchymal transition). Ero1, a protein involved in clearing unfolded proteins and a member of the ER-Stress (endoplasmic reticulum-stress) pathway was also markedly reduced. Furthermore, they have found that the *MSLN* knock-down caused a significant increase in fraction of cancer cells in S-phase (Wang et al. 2012). On the contrary, for the Mero-14 cells we have observed an increase in fraction of cells in G_0-G_1 phase, in agreement with the significant stop of cell proliferation, that paralleled with the decreased expression of the PI3K/AKT survival pathway. Reassuringly *MSLN* over-expression in NCI-H28 cells showed opposite activity of PI3K/AKT pathway. In addition, to earlier described findings, this study did reveal changes not

previously described, as the morphological changes in a tridimensional contest of the spheroid cultured in the matrigel. An important and innovative result is that using RNA interference, we showed that *MSLN* depletion sensitized Mero-14 cell lines to cisplatin, and that under this treatment, they displayed an apoptotic type of cell death, and a substantial arrest of the proliferation rate. Moreover, the combination of *MSLN* siRNA (depletion) with cisplatin enhanced the apoptotic rate measured as the activation of Caspases 3 and 7 (that paralleled with the high expression of p53, and the cleavage of PARP, at protein level). Taken together these findings reveal that the inhibition of *MSLN*, as a potential novel strategy for targeting MPM, could be a very promising approach, especially in combination with standard chemotherapies.

In this regard, monoclonal antibodies against mesothelin are being evaluated for the treatment of MPM and multiple forms of cancers (Macura et al., 2012; Al-Taei et al., 2012; Kelly et al., 2012), and show great promise for clinical development for solid cancers. Antibodies against mesothelin have been shown to act via immunotoxin-based inhibition of tumor growth and induction of antibody-dependent cellular cytotoxicity (ADCC). Very recently, Tang et al., used phage display antibody engineering technology and synthetic peptide screening to identify SD1, a human single-domain antibody to mesothelin. And they have generated a recombinant human Fc (SD1-hFc) fusion protein. Interestingly, the SD1-hFc protein exhibits strong complement-dependent cytotoxicity (CDC) activity, in addition to ADCC, against mesothelin-expressing tumor cells. Furthermore, it causes growth inhibition of human tumor xenografts in nude mice as a single agent (Tang et al., 2013). Some of the therapeutic agents that target cell surface mesothelin have been evaluated in preclinical and clinical studies. Recombinant immunotoxin SS1P is composed of a variable fragment (Fv) of SS1 and a truncated form of *Pseudomonas* exotoxin A (PE) (Chowdhury et al., 1998; Chowdhury et al., 1999). Two phase I clinical trials of SS1P were completed at the U.S. National Cancer Institute (NCI) (Hassan et al., 2007; Kreitman et al., 2009). MORAb-009 (amatuximab), a chimeric (mouse/human) antibody containing murine SS1 Fv and human IgGγ1 and *k* constant regions, was developed (Hassan et al., 2007). A phase I clinical trial of MORAb-009 for MPM, pancreatic cancer and ovarian cancer patients was recently completed (Hassan et al., 2010). A total of 24 subjects were treated, including 13 MPM, 7 pancreatic cancer, and 4 ovarian cancer patients. Eleven subjects had stable disease. Phase II studies of MORAb-009 in different mesothelin-expressing cancers are ongoing.

6. Conclusions and future perspectives

The results prompt the conclusion that, keeping with the screening of RNA markers in MPM samples, we can identify a panel of MGs specific for the analyzed disease. Regarding the negative outcome of mutational analysis, we supposed that the malignancy is not driven by somatic mutations of *PDGFRB*, but it could be involved in MPM because of its increased expression (that could be functionally equivalent to a constitutive activation), rather than somatic mutations. As regard the *CALB2* depletion, from our negative finding we can hypothesize that this gene does not seem intimately linked in triggering the disease. Finally, the most important result of this work provide evidence for a possible use of *MSLN* siRNA alone or in combination with chemotherapy for the treatment of MPM.

For the future, it could be interesting adopt the same approach that we employed to study the behavior of *MSLN* and *CALB2*, also in order to analyze the other genes resulted up-regulated in the MPM. This disease is rare, and for this reason, acquiring a large set of sample results very difficult. This represents a strong challenge that could allow to further validate our finding in an independent and larger number of specimens.

Appendix 1. Number of studies statistically significant with Up-regulation >1.3-fold or Down-regulation <0.7-fold

Gene name from official HUGO nomenclature	Gene name as reported in the manuscript	Rank	Total number of studies	Total number of studies with a statistically significant deregulation	MPM unspecified	MPM epithelioid	MPM sarcomatoid	MPM biphasic	Total number of studies corrected by authors	Total number of studies with a statistically significant deregulation, corrected by authors	Final judgment	P-value	Reference (1)	Reference (2)	Reference (3)
ANXA4	*ANXA4*	*1*	6	3	UUU				6	3	Up-regulated	6,85E-06	PMID:12912960	PMID:14732480	PMID:15920167
ASS1	*ASS1*	*2*	7	3	UUU				7	3	Up-regulated	1,19E-05	PMID:12912960	PMID:14732480	PMID:15920167
JUNB	*JUNB*	*3*	2	2	U	U	U		2	2	Up-regulated	4,97E-05	PMID:11251971	PMID:15136399	
S100A11	*S100A11*	*3*	2	2	UU				2	2	Up-regulated	4,97E-05	PMID:15920167	PMID:12912960	
PTGS2	*PTGS2*	*3*	2	2	DD				2	2	Down-regulated	4,97E-05	PMID:12912960	PMID:15920167	
TMEM176A	*HCA112*	*3*	2	2	UU				2	2	Up-regulated	4,97E-05	PMID:14732480	PMID:15920167	
HEG1	*KIAA1237*	*3*	2	2	UU				2	2	Up-regulated	4,97E-05	PMID:14732480	PMID:15920167	
RCN2	*RCN2*	*3*	2	2	UU				2	2	Up-regulated	4,97E-05	PMID:12912960	PMID:15920167	
KIF23	*KIF23*	*3*	3	3	UUU				2	2	Up-regulated	4,97E-05	PMID:19753302	PMID:19380173	PMID:19662092
PLK2	*PLK2*	*10*	3	2	UU				3	2	Up-regulated	1,48E-04	PMID:11034307	PMID:15920167	
UBXN4	*UBXN4*	*10*	3	2	U	U	U		3	2	Up-regulated	1,48E-04	PMID:15136399	PMID:15920167	
DSP	*DSP*	*10*	3	2	UU				3	2	Up-regulated	1,48E-04	PMID:12912960	PMID:15920167	
EEF2	*EEF2*	*10*	3	2	UU				3	2	Up-regulated	1,48E-04	PMID:12912960	PMID:15920167	
FGF9	*FGF9*	*10*	3	2	UU				3	2	Up-regulated	1,48E-04	PMID:14732480	PMID:15920167	
HELLS	*HELLS*	*10*	3	2	UU				3	2	Up-regulated	1,48E-04	PMID:19753302	PMID:19662092	
SULF1	*KIAA1077*	*10*	3	2	UU				3	2	Up-regulated	1,48E-04	PMID:14732480	PMID:15920167	
KIF14	*KIF14*	*10*	3	2	UU				3	2	Up-regulated	1,48E-04	PMID:19753302	PMID:19380173	
NMU	*NMU*	*10*	3	2	UU				3	2	Up-regulated	1,48E-04	PMID:14732480	PMID:15920167	
CENPF	*CENPF*	*10*	4	3	UUU				3	2	Up-regulated	1,48E-04	PMID:19753302	PMID:19380173	PMID:19662092
TOP2A	*TOP2A*	*10*	4	3	UUU				3	2	Up-regulated	1,48E-04	PMID:19753302	PMID:19380173	PMID:19662092
BUB1	*BUB1*	*10*	4	3	UUU				3	2	Up-regulated	1,48E-04	PMID:19753302	PMID:19380173	PMID:19662092
CDK2AP1	*CDK2AP1*	*10*	4	3	UUU				3	2	Up-regulated	1,48E-04	PMID:19380173	PMID:19662092	PMID:15920167
RAD21	*RAD21*	*10*	4	3	UUU				3	2	Up-regulated	1,48E-04	PMID:19380173	PMID:19662092	PMID:15920167
SSBP1	*SSBP1*	*10*	4	3	UUU				3	2	Up-regulated	1,48E-04	PMID:19380173	PMID:19662092	PMID:15920167
MCM2	*MCM2*	*10*	4	3	UUU				3	2	Up-regulated	1,48E-04	PMID:19753302	PMID:19380173	PMID:19662092
FGF2	*FGF2*	*26*	4	2	U	U	U		4	2	Up-regulated	2,94E-04	PMID:15136399	PMID:11034307	
SMC4	*SMC4*	*26*	4	2	UU				4	2	Up-regulated	2,94E-04	PMID:19662092	PMID:15920167	
CDC2	*CDC2*	*26*	5	3	UUU				4	2	Up-regulated	2,94E-04	PMID:19753302	PMID:19380173	PMID:19662092
ACSL1	*ACSL1*	*26*	4	2	DD				4	2	Down-regulated	2,94E-04	PMID:19753302	PMID:15920167	
AURKA	*AURKA*	*26*	5	3	UUU				4	2	Up-regulated	2,94E-04	PMID:19753302	PMID:19380173	PMID:19662092
BIRC5	*BIRC5*	*26*	5	3	UUU				4	2	Up-regulated	2,94E-04	PMID:19753302	PMID:19380173	PMID:19662092
MKI67	*MKI67*	*26*	4	2	UU				4	2	Up-regulated	2,94E-04	PMID:19753302	PMID:19380173	
AOC3	*AOC3*	*26*	4	2	DD				4	2	Down-regulated	2,94E-04	PMID:19753302	PMID:15920167	
PPARA	*PPARA*	*26*	4	2	DD				4	2	Down-regulated	2,94E-04	PMID:19753302	PMID:19662092	
TGFBR2	*TGFBR2*	*26*	4	2	DD				4	2	Down-regulated	2,94E-04	PMID:19662092	PMID:15920167	
TSPAN7	*TSPAN7*	*26*	4	2	DD				4	2	Down-regulated	2,94E-04	PMID:19753302	PMID:15920167	
CCNO	*CCNO*	*26*	4	2	UU				4	2	Up-regulated	2,94E-04	PMID:19380173	PMID:14732480	
FANCI	*FANCI*	*26*	4	2	UU				4	2	Up-regulated	2,94E-04	PMID:19753302	PMID:19380173	
RAN	*RAN*	*26*	5	3	UUU				4	2	Up-regulated	2,94E-04	PMID:19380173	PMID:19662092	PMID:15920167
CCNB2	*CCNB2*	*26*	5	3	UUU				4	2	Up-regulated	2,94E-04	PMID:19753302	PMID:19380173	PMID:19662092
SMARCA4	*SMARCA4*	*41*	5	2	UU				5	2	Up-regulated	4,86E-04	PMID:19753302	PMID:15920167	
PTMS	*PTMS*	*41*	6	3	UUU				5	2	Up-regulated	4,86E-04	PMID:19380173	PMID:12912960	PMID:19662092
CHEK1	*CHEK1*	*41*	5	2	UU				5	2	Up-regulated	4,86E-04	PMID:19753302	PMID:19380173	
CAV1	*CAV1*	*41*	5	2	DD				5	2	Down-regulated	4,86E-04	PMID:19662092	PMID:15920167	
NR4A2	*NR4A2*	*41*	5	2	DD				5	2	Down-regulated	4,86E-04	PMID:19662092	PMID:15920167	
PECAM1	*PECAM1*	*41*	5	2	DD				5	2	Down-regulated	4,86E-04	PMID:19753302	PMID:15920167	
SFRP1	*SFRP1*	*41*	5	2	DD				5	2	Down-regulated	4,86E-04	PMID:12912960	PMID:15920167	
TACC1	*TACC1*	*41*	5	2	DD				5	2	Down-regulated	4,86E-04	PMID:19753302	PMID:15920167	
VWF	*VWF*	*41*	5	2	DD				5	2	Down-regulated	4,86E-04	PMID:19753302	PMID:15920167	
IFITM1	*IFITM1*	*41*	5	2	UU				5	2	Up-regulated	4,86E-04	PMID:14732480	PMID:15920167	
LGALS3BP	*LGALS3BP*	*41*	5	2	UU				5	2	Up-regulated	4,86E-04	PMID:12912960	PMID:15920167	
PTGIS	*PTGIS*	*41*	5	2	UU				5	2	Up-regulated	4,86E-04	PMID:14732480	PMID:15920167	
S100A10	*S100A10*	*41*	5	2	UU				5	2	Up-regulated	4,86E-04	PMID:12912960	PMID:15920167	
XPOT	*XPOT*	*41*	5	2	UU				5	2	Up-regulated	4,86E-04	PMID:19380173	PMID:15920167	
NME2	*NME2*	*41*	6	3	UUU				5	2	Up-regulated	4,86E-04	PMID:19380173	PMID:15920167	PMID:19662092
COL1A1	*COL1A1*	*56*	6	2	UU				6	2	Up-regulated	7,24E-04	PMID:14732480	PMID:15920167	
CDK4	*CDK4*	*56*	6	2	UU				6	2	Up-regulated	7,24E-04	PMID:15920167	PMID:20043850	
CCNB1	*CCNB1*	*56*	7	3	UUU				6	2	Up-regulated	7,24E-04	PMID:19753302	PMID:19380173	PMID:19662092
EGR2	*EGR2*	*56*	6	2	DD				6	2	Down-regulated	7,24E-04	PMID:19662092	PMID:15920167	
EGR3	*EGR3*	*56*	6	2	DD				6	2	Down-regulated	7,24E-04	PMID:19662092	PMID:15920167	
EPAS1	*EPAS1*	*56*	6	2	DD				6	2	Down-regulated	7,24E-04	PMID:19753302	PMID:15920167	
ALDOA	*ALDOA*	*56*	6	2	UU				6	2	Up-regulated	7,24E-04	PMID:12912960	PMID:15920167	
CFB	*BF*	*56*	6	2	UU				6	2	Up-regulated	7,24E-04	PMID:14732480	PMID:15920167	
CCT2	*CCT2*	*56*	6	2	UU				6	2	Up-regulated	7,24E-04	PMID:19380173	PMID:15920167	
CDH11	*CDH11*	*56*	6	2	UU				6	2	Up-regulated	7,24E-04	PMID:14732480	PMID:15920167	
COL6A1	*COL6A1*	*56*	6	2	UU				6	2	Up-regulated	7,24E-04	PMID:19753302	PMID:15920167	
CRIP1	*CRIP1*	*56*	6	2	UU				6	2	Up-regulated	7,24E-04	PMID:14732480	PMID:15920167	
EIF4G1	*EIF4G1*	*56*	6	2	UU				6	2	Up-regulated	7,24E-04	PMID:12912960	PMID:14732480	
FHL1	*FHL1*	*56*	6	2	UU				6	2	Up-regulated	7,24E-04	PMID:14732480	PMID:15920167	
KIF5B	*KIF5B*	*56*	6	2	UU				6	2	Up-regulated	7,24E-04	PMID:19380173	PMID:15920167	
MSLN	*MSLN*	*56*	6	2	UU				6	2	Up-regulated	7,24E-04	PMID:14732480	PMID:15920167	
PGM1	*PGM1*	*56*	6	2	UU				6	2	Up-regulated	7,24E-04	PMID:14732480	PMID:15920167	
PKM2	*PKM2*	*56*	6	2	UU				6	2	Up-regulated	7,24E-04	PMID:19662092	PMID:15920167	
PSMD11	*PSMD11*	*56*	6	2	UU				6	2	Up-regulated	7,24E-04	PMID:19380173	PMID:14732480	
VCAN	*VCAN*	*56*	6	2	UU				6	2	Up-regulated	7,24E-04	PMID:14732480	PMID:15920167	
CDK7	*CDK7*	*56*	7	3	UUU				6	2	Up-regulated	7,24E-04	PMID:19380173	PMID:11034307	PMID:19662092
PCNA	*PCNA*	*56*	7	3	UUU				6	2	Up-regulated	7,24E-04	PMID:19380173	PMID:11034307	PMID:19662092
GAPDH	*GAPDH*	*56*	6	2	UU				6	2	Up-regulated	7,24E-04	PMID:12912960	PMID:15920167	
ITGA4	*ITGA4*	*79*	8	3	UUU				7	2	Up-regulated	1,01E-03	PMID:12912960	PMID:11034307	PMID:14732480
ANK2	*ANK2*	*79*	7	2	DD				7	2	Down-regulated	1,01E-03	PMID:19753302	PMID:15920167	
COL11A1	*COL11A1*	*79*	7	2	UU				7	2	Up-regulated	1,01E-03	PMID:14732480	PMID:15920167	
PGK1	*PGK1*	*79*	7	2	UU				7	2	Up-regulated	1,01E-03	PMID:12912960	PMID:15920167	
TNPO2	*TNPO2*	*79*	7	2	UU				7	2	Up-regulated	1,01E-03	PMID:12912960	PMID:11034307	
CCNH	*CCNH*	*84*	8	2	U	U	U	U	8	2	Up-regulated	1,33E-03	PMID:15136399	PMID:11034307	
KRT18	*KRT18*	*84*	8	2	UU				8	2	Up-regulated	1,33E-03	PMID:12912960	PMID:15920167	
KRT5	*KRT5*	*84*	8	2	UU				8	2	Up-regulated	1,33E-03	PMID:12912960	PMID:15920167	
SOD1	*SOD1*	*84*	8	2	UU				8	2	Up-regulated	1,33E-03	PMID:12912960	PMID:11034307	
CLDN15	*CLDN15*	*88*	1	1	U				1	1	Up-regulated	7,05E-03	PMID:15920167		
BLMH	*BLMH*	*88*	2	2	UU				1	1	Up-regulated	7,05E-03	PMID:11034307	PMID:14732480	
ITGBL1	*ITGBL1*	*88*	2	2	UU				1	1	Up-regulated	7,05E-03	PMID:11034307	PMID:14732480	
C21orf7	*c21ORF7*	*88*	1	1		D	D	D	1	1	Down-regulated	7,05E-03	PMID:15136399		
CCNF	*CCNF*	*88*	1	1	D				1	1	Down-regulated	7,05E-03	PMID:12912960		
DSC3	*DSC3*	*88*	1	1	D				1	1	Down-regulated	7,05E-03	PMID:11251971		
TNC	*HXB*	*88*	1	1	D				1	1	Down-regulated	7,05E-03	PMID:11251971		
OSTF1	*OSTF1*	*88*	1	1	D				1	1	Down-regulated	7,05E-03	PMID:12912960		
PDGFRA	*PDGFRA*	*88*	1	1	D				1	1	Down-regulated	7,05E-03	PMID:11251971		
RNASEL	*RNASEL*	*88*	1	1	D				1	1	Down-regulated	7,05E-03	PMID:11034307		
ST8SIA5	*ST8SIA5*	*88*	1	1	D				1	1	Down-regulated	7,05E-03	PMID:12912960		
DAP	*DAP*	*88*	1	1	U				1	1	Up-regulated	7,05E-03	PMID:12912960		
TMSB4X	*AA634103*	*88*	1	1	U				1	1	Up-regulated	7,05E-03	PMID:12912960		
ACOX1	*ACOX1*	*88*	1	1		U	U	U	1	1	Up-regulated	7,05E-03	PMID:15136399		

MLLT10	*AF10*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
ARMCX2	*ALEX2*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
PRDX3	*AOP1*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
FAS	*APO-1*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:12912960
ARPC1B	*ARC41*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:12912960
RHOB	*ARHB*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
ASPA	*ASPA*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
CDH19	*CDH19*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
CNTN1	*CNTN1*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
COL4A1	*COL4A1*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
ERBB4	*ERBB4*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:11251971
ACSL6	*FACL6*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
FGF3	*FGF3*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:11251971
STAT3	*FLJ20882*	*88*	1	1		U	U			1	1	Up-regulated	7,05E-03	PMID:15136399
HLA-DRB3	*HLA-DRB3*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
HSPH1	*HSP105B*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
AUTS2	*KIAA0442*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
L1CAM	*L1CAM*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:11251971
LAPTM5	*LAPTM5*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
LBP	*LBP*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
PDF	*LOC64146*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
NFKBIZ	*MAIL*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
METAP1	*METAP1*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
NTF3	*NgF2*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:11251971
SERPINB2	*PAI-2*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:11034307
PDGFRB	*PDGFRB*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:11251971
PGRMC1	*PGRMC1*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
PSMD3	*PSMD3*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
RHO	*RHO*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:12912960
FIG4	*SAC3*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
SFRP2	*SARP1*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:11251971
NPTN	*SDFR1*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
SPI1	*SPI1*	*88*	1	1		U	U			1	1	Up-regulated	7,05E-03	PMID:15136399
TOP2B	*TOP2B*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:14732480
ZNF630	*ZNF630*	*88*	1	1			U	U		1	1	Up-regulated	7,05E-03	PMID:15136399
MACF1	*ACF7*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
ADAMTS1	*ADAMTS1*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
PLIN2	*ADFP*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
ALDH1B1	*ALDH1B1*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
ALOX5AP	*ALOX5AP*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
C10orf116	*APM2*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
ATP1A2	*ATP1A2*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
ATP6V0E1	*ATP6H*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
MALL	*BENE*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
CD93	*C1QR1*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
C5AR1	*C5R1*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
CDC5L	*CDC5L*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
CD52	*CDW52*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
CEACAM6	*CEACAM6*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
CH25H	*CH25H*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
CTSS	*CTSS*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
CD55	*DAF*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
C10orf10	*DEPP*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
SYNM	*DMN*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
RCAN1	*DSCR1*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
F3	*F3*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
FBLN1	*FBLN1*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
ZC3HAV1	*FLB6421*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
LPCAT1	*FLJ12443*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
TCTN2	*FLJ12975*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
C1orf115	*FLJ14146*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
C1orf56	*FLJ20519*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
CIDEC	*FLJ20871*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
C14orf139	*FLJ21276*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
PHF17	*FLJ22479*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
C4orf31	*FLJ23191*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
GEM	*GEM*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
GTPBP3	*GTPBG3*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
HLA-DRB4	*HLA-DRB4*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
IRF8	*ICSBP1*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
IGL@	*IGL@*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
IGLJ3	*IGLJ3*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
LAMP3	*LAMP3*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
PLAC8	*LOC51316*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
LMO3	*LOC55885*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
PNO1	*LOC56902*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
ITIH5	*MGC10848*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
C17orf91	*MGC14376*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
LOH3CR2A	*NAG-7*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
TSPAN13	*NET-6*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
NAMPT	*PBEF*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
PCOLCE2	*PCOLCE2*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
LITAF	*PIG7*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
PIK3R3	*PIK3R3*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
PLIN1	*PLIN*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
CAMK2N1	*PRO1489*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
RASSF2	*RASSF2*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
C13orf15	*RGC32*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
S100A8	*S100A8*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
S100A9	*S100A9*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
CCL15	*SCYA15*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
CCL18	*SCYA18*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
CXCL14	*SCYB14*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
SERPINA3	*SERPINA3*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
SFTPB	*SFTPB*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
SFTPD	*SFTPD*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
SORBS1	*SH3D5*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
HOPX	*SMAP31*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
SOCS2	*STAT12*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
TSPAN8	*TM4SF3*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
SCGB1A1	*UGB*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
LYVE1	*XLKD1*	*88*	1	1	D					1	1	Down-regulated	7,05E-03	PMID:15920167
ADAM10	*ADAM10*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
ADNP	*ADNP*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
C11orf9	*C11orf9*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
CPA4	*CPA4*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
EID1	*CRI1*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
CRIM1	*CRIM1*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
MYOF	*FER1L3*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
ZNF532	*FLJ10697*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
MAP4K4	*FLJ20373*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
DDIT4	*FLJ20500*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
SLC12A8	*FLJ23188*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
ZFPM2	*FOG2*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
HIST1H4C	*H4FG*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
C19orf53	*HSPC023*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
TRMT112	*HSPC152*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
WWC1	*KIAA0869*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
MEMO1	*LOC51072*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
WBP5	*LOC51186*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
TMEM176B	*LR8*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
LRRC8D	*LRRC5*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
NDFIP1	*MGC10924*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
TMEM106C	*MGC5576*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
MRPL3	*MRPL3*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
NSUN5	*NOL1R*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
NSUN5C	*NOL1R2*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
PABPC3	*PABPC3*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
SERBP1	*PAI-RBP1*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167
PIGCP1	*PIGCP1*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167

PNMA2	*PNMA2*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167		
C19orf10	*R33729_1*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167		
SEC61A1	*SEC61A1*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167		
SGPL1	*SGPL1*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167		
PRSS23	*SPUVE*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167		
HSPA13	*STCH*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167		
TNS3	*TEM6*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167		
CNPY2	*TMEM4*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167		
UBL5	*UBL5*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167		
FBXO11	*VIT1*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:15920167		
DTL	*DTL*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:19753302		
NDC80	*NDC80*	*88*	1	1	U					1	1	Up-regulated	7,05E-03	PMID:19380173		
PTPRF	*PTPRF*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
UPK1B	*UPK1B*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
THBS2	*THBS2*	*244*	3	2	UU					2	1	Up-regulated	1,40E-02	PMID:11034307	PMID:14732480	
CTSH	*CTSH*	*244*	2	1	D					2	1	Down-regulated	1,40E-02	PMID:11034307		
GDF10	*GDF10*	*244*	2	1	D					2	1	Down-regulated	1,40E-02	PMID:12912960		
HTRA1	*HTRA1*	*244*	2	1	D					2	1	Down-regulated	1,40E-02	PMID:11034307		
KLF9	*BTEB1*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
BYSL	*BYSL*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
COL4A4	*COL4A4*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:12912960		
FGL2	*FGL2*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
FMOD	*FMOD*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
HLA-DOB	*HLA-DOB*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
HLA-DPB1	*HLA-DPB1*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
HLA-DRB1	*HLA-DRB1*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
HNRNPC	*HNRPC*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
IL4R	*IL4R*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
PSMD6	*KIAA0107*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
TTC9	*KIAA0227*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
KIAA0247	*KIAA0247*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
SUSD5	*KIAA0527*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
KIAA0528	*KIAA0528*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
COBLL1	*COBLL1*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
LRRC1	*LRRC1*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
MAP1B	*MAP1B*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
UPK3B	*UPK3B*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
MTMR6	*MTMR6*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
IL32	*NK4*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
OR7E12P	*OR7E12P*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
RARS	*RARS*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:11034307		
RND3	*RND3*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:11034307		
RSC1A1	*RSC1A1*	*244*	2	1			U			2	1	Up-regulated	1,40E-02	PMID:15136399		
SPRR2A	*SPRR2A*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
TAF15	*TAF2N*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
VAMP8	*VAMP8*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
ZNF277	*ZNF277*	*244*	2	1		U	U	U		2	1	Up-regulated	1,40E-02	PMID:15136399		
GCLC	*GCLC*	*244*	3	2	UU					2	1	Up-regulated	1,40E-02	PMID:11034307	PMID:14732480	
ABCA8	*ABCA8*	*244*	2	1	D					2	1	Down-regulated	1,40E-02	PMID:15920167		
AREG	*AREG*	*244*	2	1	D					2	1	Down-regulated	1,40E-02	PMID:15920167		
CD83	*CD83*	*244*	2	1	D					2	1	Down-regulated	1,40E-02	PMID:15920167		
CPA3	*CPA3*	*244*	2	1	D					2	1	Down-regulated	1,40E-02	PMID:15920167		
EMP2	*EMP2*	*244*	2	1	D					2	1	Down-regulated	1,40E-02	PMID:15920167		
STOM	*EPB72*	*244*	2	1	D					2	1	Down-regulated	1,40E-02	PMID:15920167		
INPP5A	*INPP5A*	*244*	2	1	D					2	1	Down-regulated	1,40E-02	PMID:15920167		
KDR	*KDR*	*244*	2	1	D					2	1	Down-regulated	1,40E-02	PMID:11251971		
LHFP	*LHFP*	*244*	2	1	D					2	1	Down-regulated	1,40E-02	PMID:15920167		
MAN1A1	*MAN1A1*	*244*	2	1	D					2	1	Down-regulated	1,40E-02	PMID:15920167		
MEF2C	*MEF2C*	*244*	2	1	D					2	1	Down-regulated	1,40E-02	PMID:15920167		
MEFV	*MEFV*	*244*	2	1	D					2	1	Down-regulated	1,40E-02	PMID:15920167		
OPHN1	*OPHN1*	*244*	2	1	D					2	1	Down-regulated	1,40E-02	PMID:15920167		
PBK	*PBK*	*244*	2	1		D	D	D		2	1	Down-regulated	1,40E-02	PMID:15136399		
SELE	*SELE*	*244*	2	1	D					2	1	Down-regulated	1,40E-02	PMID:15920167		
TACSTD2	*TACSTD2*	*244*	2	1	D					2	1	Down-regulated	1,40E-02	PMID:15920167		
UNG	*UNG*	*244*	2	1	D					2	1	Down-regulated	1,40E-02	PMID:12912960		
ABCE1	*ABCE1*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:11034307		
AP3S1	*AP3S1*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
ARHGAP4	*ARHGAP4*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:11251971		
BET1	*BET1*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
FAM120A	*C9orf10*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
COL4A5	*COL4A5*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
COL5A2	*COL5A2*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
KLF6	*COPEB*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
CTSK	*CTSK*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
DCTD	*DCTD*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
EIF3C	*EIF3S8*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
PDIA4	*ERP70*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
FCGR3A	*FCGR3A*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
FMR1	*FMR1*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
GFPT2	*GFPT2*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
HIST1H2BG	*H2BFA*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
HIST1H2BD	*H2BFB*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
HLA-DOA	*HLA-DOA*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
HLA-DPA1	*HLA-DPA1*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
HSP90B1	*HSP90B1*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:12912960		
HSPA6	*HSPA6*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
IL18	*IL18*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
KARS	*KARS*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:11034307		
PDZD2	*KIAA0300*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
LAPTM4B	*LC27*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
PDLIM5	*LIM*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
LUC7L3	*LUC7A*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
CAPRIN1	*M11S1*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
MDH2	*MDH*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:12912960		
NEO1	*NEO1*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
HYOU1	*ORP150*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
C5orf13	*P311*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
POLR3C	*POLR3C*	*244*	2	1			U			2	1	Up-regulated	1,40E-02	PMID:15136399		
PROCR	*PROCR*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
PTS	*PTS*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
IPO7	*RANBP7*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
RARRES1	*RARRES1*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
RBP1	*RBP1*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
RPS5	*RPS5*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
SLPI	*SLPI*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:14732480		
SOX15	*SOX15*	*244*	2	1		U	U	U		2	1	Up-regulated	1,40E-02	PMID:15136399		
SPOCK2	*SPOCK2*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:12912960		
SFRS2B	*SRP46*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
ABI1	*SSH3BP1*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
EZR	*VIL2*	*244*	2	1	U					2	1	Up-regulated	1,40E-02	PMID:15920167		
DNA2	*DNA2L*	*244*	3	2	UU					2	1	Up-regulated	1,40E-02	PMID:19380173	PMID:19662092	
ESPL1	*ESPL1*	*244*	3	2	UU					2	1	Up-regulated	1,40E-02	PMID:19380173	PMID:19662092	
GINS1	*GINS1*	*244*	3	2	UU					2	1	Up-regulated	1,40E-02	PMID:19380173	PMID:19662092	
RNASEH2A	*RNASEH2A*	*244*	3	2	UU					2	1	Up-regulated	1,40E-02	PMID:19380173	PMID:19662092	
TIMELESS	*TIMELESS*	*244*	3	2	UU					2	1	Up-regulated	1,40E-02	PMID:19380173	PMID:19662092	
POLR2J	*POLR2J*	*244*	2	1	D					2	1	Down-regulated	1,40E-02	PMID:12912960		
KRT15	*KRT15*	*348*	3	1	U					3	1	Up-regulated	2,08E-02	PMID:14732480		
NSUN2	*NSUN2*	*348*	3	1	U					3	1	Up-regulated	2,08E-02	PMID:19380173		
FEN1	*FEN1*	*348*	4	2	UU					3	1	Up-regulated	2,08E-02	PMID:19380173	PMID:19662092	
COL11A2	*COL11A2*	*348*	3	1	U					3	1	Up-regulated	2,08E-02	PMID:12912960		
COL16A1	*COL16A1*	*348*	3	1	U					3	1	Up-regulated	2,08E-02	PMID:14732480		
CSF1R	*CSF1R*	*348*	3	1	U					3	1	Up-regulated	2,08E-02	PMID:14732480		
IFI35	*IFI35*	*348*	3	1		U	U			3	1	Up-regulated	2,08E-02	PMID:15136399		
ITGA1	*ITGA1*	*348*	3	1	U					3	1	Up-regulated	2,08E-02	PMID:14732480		
ITGA3	*ITGA3*	*348*	3	1	U					3	1	Up-regulated	2,08E-02	PMID:11034307		
PLLP	*PLLP*	*348*	3	1	U					3	1	Up-regulated	2,08E-02	PMID:15920167		
PRKY	*PRKY*	*348*	3	1			U	U		3	1	Up-regulated	2,08E-02	PMID:15136399		
COPS2	*TRIP15*	*348*	3	1	U					3	1	Up-regulated	2,08E-02	PMID:15920167		
ORC6L	*ORC6L*	*348*	4	2	UU					3	1	Up-regulated	2,08E-02	PMID:19662092	PMID:19380173	
NUSAP1	*NUSAP1*	*348*	4	2	UU					3	1	Up-regulated	2,08E-02	PMID:19662092	PMID:19380173	

GADD45A	*GADD45A*	*348*	4	2	UU	3	1	Up-regulated	2,08E-02	PMID:11034307	PMID:14732480	
RPS6KA5	*RPS6KA5*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:19753302		
S1PR1	*S1PR1*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:19753302		
CSF2RB	*CSF2RB*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:19662092		
EMP1	*EMP1*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:15920167		
GSTM1	*GSTM1*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:19662092		
HOXA5	*HOXA5*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:19753302		
GPD1L	*KIAA0089*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:15920167		
RP11-159J2.1	*KIAA0603*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:15920167		
GPR116	*KIAA0758*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:15920167		
LDLR	*LDLR*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:15920167		
MGST2	*MGST2*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:15920167		
MNDA	*MNDA*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:15920167		
NFIL3	*NFIL3*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:15920167		
PGAP3	*PER1*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:19662092		
PER3	*PER3*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:19662092		
PGC	*PGC*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:15920167		
PGRMC2	*PGRMC2*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:19662092		
PRKAR2B	*PRKAR2B*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:15920167		
RNASE4	*RNASE4*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:15920167		
SPN	*SPN*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:15920167		
SYNPO2	*SYNPO2*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:19753302		
THBD	*THBD*	*348*	3	1	D	3	1	Down-regulated	2,08E-02	PMID:15920167		
AATF	*AATF*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19380173		
ACTR3	*ACTR3*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:15920167		
AGGF1	*AGGF1*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19662092		
BCCIP	*BCCIP*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19380173		
CD53	*CD53*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:14732480		
CHD1	*CHD1*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:14732480		
CKAP2	*CKAP2*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19753302		
COL5A1	*COL5A1*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:15920167		
CTSL1	*CTSL*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:12912960		
DLGAP5	*DLG7*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19753302		
DYNC1H1	*DYNC1H1*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19380173		
E2F7	*E2F7*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19662092		
EME1	*EME1*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19380173		
F10	*F10*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:14732480		
FAAH	*FAAH*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:14732480		
FANCD2	*FANCD2*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19380173		
FANCF	*FANCF*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19380173		
FICD	*FICD*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19380173		
GFPT1	*GFPT1*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:15920167		
FAM3C	*GS3786*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:15920167		
HIST2H2AA3	*H2AFO*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:15920167		
HLA-DMB	*HLA-DMB*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:14732480		
HMGB2	*HMG2*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:15920167		
INHBE	*INHBE*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19662092		
CLINT1	*KIAA0171*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:15920167		
SR140	*KIAA0332*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:15920167		
TBC1D9	*KIAA0882*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:15920167		
KIF18A	*KIF18A*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19380173		
LAMA5	*LAMA5*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19753302		
MARCKS	*MACS*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:15920167		
NCAPD3	*NCAPD3*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19662092		
NRCAM	*NRCAM*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:14732480		
NSMCE1	*NSMCE1*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19380173		
P4HB	*P4HB*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:15920167		
PPL	*PPL*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:15920167		
RAB2A	*RAB2*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:15920167		
RAD18	*RAD18*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19380173		
RAD54L	*RAD54L*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19380173		
RCC2	*RCC2*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19662092		
TMED2	*RNP24*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:15920167		
SEMA3C	*SEMA3C*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:15920167		
SHFM1	*SHFM1*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19380173		
SOX4	*SOX4*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19753302		
STK36	*STK36*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19753302		
TCF19	*TCF19*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19380173		
TAX1BP3	*TIP-1*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:15920167		
TRNT1	*TRNT1*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19380173		
UHRF1	*UHRF1*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19380173		
VTN	*VTN*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:15920167		
XPO5	*XPO5*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:19380173		
CDC23	*CDC23*	*348*	4	2	UU	3	1	Up-regulated	2,08E-02	PMID:19662092	PMID:19380173	
RFC4	*RFC4*	*348*	4	2	UU	3	1	Up-regulated	2,08E-02	PMID:19380173	PMID:19662092	
RFC5	*RFC5*	*348*	4	2	UU	3	1	Up-regulated	2,08E-02	PMID:19380173	PMID:19662092	
CLASP1	*CLASP1*	*348*	4	2	UU	3	1	Up-regulated	2,08E-02	PMID:19380173	PMID:19662092	
GINS2	*GINS2*	*348*	4	2	UU	3	1	Up-regulated	2,08E-02	PMID:19380173	PMID:19662092	
GLI2	*GLI2*	*348*	4	2	UU	3	1	Up-regulated	2,08E-02	PMID:19380173	PMID:19662092	
GMNN	*GMNN*	*348*	4	2	UU	3	1	Up-regulated	2,08E-02	PMID:19380173	PMID:19662092	
KIF2C	*KIF2C*	*348*	4	2	UU	3	1	Up-regulated	2,08E-02	PMID:19380173	PMID:19662092	
MCM3	*MCM3*	*348*	4	2	UU	3	1	Up-regulated	2,08E-02	PMID:19380173	PMID:19662092	
MCM4	*MCM4*	*348*	4	2	UU	3	1	Up-regulated	2,08E-02	PMID:19662092	PMID:19380173	
MCM6	*MCM6*	*348*	4	2	UU	3	1	Up-regulated	2,08E-02	PMID:19380173	PMID:19662092	
NUDC	*NUDC*	*348*	4	2	UU	3	1	Up-regulated	2,08E-02	PMID:19380173	PMID:19662092	
PRIM2	*PRIM2*	*348*	4	2	UU	3	1	Up-regulated	2,08E-02	PMID:19380173	PMID:19662092	
RBM14	*RBM14*	*348*	4	2	UU	3	1	Up-regulated	2,08E-02	PMID:19380173	PMID:19662092	
HDGF	*HDGF*	*348*	3	1	U	3	1	Up-regulated	2,08E-02	PMID:15920167		
TACC2	*TACC2*	*450*	4	1	D	4	1	Down-regulated	2,76E-02	PMID:15920167		
KIAA1199	*KIAA1199*	*450*	4	1	U	4	1	Up-regulated	2,76E-02	PMID:15920167		
LGALS8	*LGALS8*	*450*	4	1	U	4	1	Up-regulated	2,76E-02	PMID:15920167		
ITGA2	*ITGA2*	*450*	4	1	U	4	1	Up-regulated	2,76E-02	PMID:12912960		
PLP2	*PLP2*	*450*	4	1	U	4	1	Up-regulated	2,76E-02	PMID:15920167		
PPAPDC1B	*PPAPDC1B*	*450*	3	1	U	4	1	Up-regulated	2,76E-02	PMID:19380173		
MARS	*MARS*	*450*	4	1	U	4	1	Up-regulated	2,76E-02	PMID:19380173		
UBE2C	*UBE2C*	*450*	4	1	U	4	1	Up-regulated	2,76E-02	PMID:19753302		
BUB1B	*BUB1B*	*450*	5	2	UU	4	1	Up-regulated	2,76E-02	PMID:19380173	PMID:19662092	
GBA2	*GBA2*	*450*	4	1	D	4	1	Down-regulated	2,76E-02	PMID:12912960		
HLF	*HLF*	*450*	4	1	D	4	1	Down-regulated	2,76E-02	PMID:19662092		
TXNL4A	*TXNL4A*	*450*	4	1	U	4	1	Up-regulated	2,76E-02			
CDC27	*CDC27*	*450*	4	1	U	4	1	Up-regulated	2,76E-02	PMID:14732480		
FSTL3	*FSTL3*	*450*	4	1	U	4	1	Up-regulated	2,76E-02	PMID:19753302		
KIF4A	*KIF4A*	*450*	4	1	U	4	1	Up-regulated	2,76E-02	PMID:19753302		
KRT14	*KRT14*	*450*	4	1	U	4	1	Up-regulated	2,76E-02	PMID:14732480		
KRT7	*KRT7*	*450*	4	1	U	4	1	Up-regulated	2,76E-02	PMID:12912960		
LAMC2	*LAMC2*	*450*	4	1	U	4	1	Up-regulated	2,76E-02	PMID:19753302		
MMP14	*MMP14*	*450*	4	1	U	4	1	Up-regulated	2,76E-02	PMID:19753302		
RPSA	*RPSA*	*450*	4	1	U	4	1	Up-regulated	2,76E-02	PMID:12912960		
TIA1	*TIA1*	*450*	4	1	U	4	1	Up-regulated	2,76E-02	PMID:15920167		
TPBG	*TPBG*	*450*	4	1	U	4	1	Up-regulated	2,76E-02	PMID:15920167		
TRAF4	*TRAF4*	*450*	4	1	U	4	1	Up-regulated	2,76E-02	PMID:19753302		
UBE2V2	*UBE2V2*	*450*	4	1	U	4	1	Up-regulated	2,76E-02	PMID:19380173		
ADH1B	*ADH1B*	*450*	4	1	D	4	1	Down-regulated	2,76E-02	PMID:15920167		
AGTR1	*AGTR1*	*450*	4	1	D	4	1	Down-regulated	2,76E-02	PMID:19753302		
ALDH1A1	*ALDH1A1*	*450*	4	1	D	4	1	Down-regulated	2,76E-02	PMID:15920167		
AQP3	*AQP3*	*450*	4	1	D	4	1	Down-regulated	2,76E-02	PMID:15920167		
ARHGEF3	*ARHGEF3*	*450*	4	1	D	4	1	Down-regulated	2,76E-02	PMID:19753302		
ATP5J	*ATP5J*	*450*	4	1	D	4	1	Down-regulated	2,76E-02	PMID:19753302		
CCL14	*CCL14*	*450*	4	1	D	4	1	Down-regulated	2,76E-02	PMID:19753302		
CFD	*CFD*	*450*	4	1	D	4	1	Down-regulated	2,76E-02	PMID:15920167		
CLEC3B	*CLEC3B*	*450*	4	1	D	4	1	Down-regulated	2,76E-02	PMID:15920167		
COX7A1	*COX7A1*	*450*	4	1	D	4	1	Down-regulated	2,76E-02	PMID:15920167		
CXCL2	*CXCL2*	*450*	4	1	D	4	1	Down-regulated	2,76E-02	PMID:15920167		
IL8RB	*CXCR2*	*450*	4	1	D	4	1	Down-regulated	2,76E-02	PMID:19753302		
CYP4B1	*CYP4B1*	*450*	4	1	D	4	1	Down-regulated	2,76E-02	PMID:15920167		
DARC	*DARC*	*450*	4	1	D	4	1	Down-regulated	2,76E-02	PMID:19753302		
FBLN5	*FBLN5*	*450*	4	1	D	4	1	Down-regulated	2,76E-02	PMID:15920167		
FTL	*FTL*	*450*	4	1	D	4	1	Down-regulated	2,76E-02	PMID:15920167		
G0S2	*G0S2*	*450*	4	1	D	4	1	Down-regulated	2,76E-02	PMID:15920167		
GADD45B	*GADD45B*	*450*	4	1	D	4	1	Down-regulated	2,76E-02	PMID:15920167		

GNG11	*GNG11*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
GPC3	*GPC3*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
GPX3	*GPX3*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
H3F3B	*H3F3B*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
HBA1	*HBA1*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
HBA2	*HBA2*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
HEY1	*HEY1*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
IDI1	*IDI1*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
IGHM	*IGHM*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
IGKC	*IGKC*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
IL8	*IL8*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
ITM2A	*ITM2A*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
JAM2	*JAM2*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
MAFF	*MAFF*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
MUC1	*MUC1*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
MYH11	*MYH11*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
MYL4	*MYL4*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
NR1D1	*NR1D1*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:19662092			
PALMD	*PALMD*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
PLCL2	*PLCL2*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:19753302			
PLXDC1	*PLXDC1*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:19662092			
PPP2R5A	*PPP2R5A*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
PTGDS	*PTGDS*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:19753302			
RBP4	*RBP4*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
RPL27	*RPL27*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
RPL27A	*RPL27A*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
RPLP2	*RPLP2*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
SERPINA1	*SERPINA1*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
SFTPA2	*SFTPA2*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
SFTPC	*SFTPC*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
SLC19A2	*SLC19A2*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
SNX15	*SNX15*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
SPARCL1	*SPARCL1*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
SPON1	*SPON1*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
SRPX	*SRPX*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
THRB	*THRB*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:19662092			
TNS1	*TNS*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
TRA@	*TRA@*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
CEP55	*CEP55*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19662092			
AASDHPPT	*AASDHPPT*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
ALDH1A2	*ALDH1A2*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
ASPM	*ASPM*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19753302			
ATP1B1	*ATP1B1*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
ATP5G2	*ATP5G2*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
ATP5J2	*ATP5J2*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
BACE2	*BACE2*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
BAT3	*BAT3*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
BBS4	*BBS4*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19380173			
C11orf10	*C11orf10*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
C4B	*C4B*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
CBS	*CBS*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19753302			
CBX3	*CBX3*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
CCDC99	*CCDC99*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19753302			
CCNL2	*CCNL2*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19753302			
CDC123	*CDC123*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19380173			
CDCA3	*CDCA3*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19753302			
CIB1	*CIB1*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19380173			
DYNC1I2	*DYNC1I2*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19380173			
DYNLRB1	*DYNLRB1*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19380173			
EZH2	*EZH2*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19753302			
FUS	*FUS*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19753302			
GALNT7	*GALNT7*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
GLS	*GLS*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
GNAS	*GNAS1*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
GPI	*GPI*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
GTPBP4	*GTPBP4*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19380173			
KIF20A	*KIF20A*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19753302			
LEF1	*LEF1*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19753302			
MAD2L1	*MAD2L1*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19753302			
MCTS1	*MCTS1*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19380173			
MYBL2	*MYBL2*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19380173			
NIPBL	*NIPBL*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19753302			
NPIP	*NPIP*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
PCDHGC5	*PCDHGC5*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
PSMA7	*PSMA7*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19380173			
PSMD14	*PSMD14*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19380173			
RACGAP1	*RACGAP1*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19753302			
RAD51AP1	*RAD51AP1*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19380173			
REC8	*REC8*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
RPL36	*RPL36*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
RUVBL1	*RUVBL1*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19662092			
SDR16C5	*SDR16C5*	*450*	4	1		U	U	U	4	1	Up-regulated	2,76E-02	PMID:15136399			
SERPINH1	*SERPINH1*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
SMC2	*SMC2*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19662092			
SPP1	*SPP1*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
SRPK1	*SRPK1*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19380173			
SULT1A1	*SULT1A1*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
TOPBP1	*TOPBP1*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
TUBB4	*TUBB4*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:15920167			
XRCC4	*XRCC4*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:19380173			
CDC20	*CDC20*	*450*	5	2	UU				4	1	Up-regulated	2,76E-02	PMID:19380173	PMID:19662092		
MSH6	*MSH6*	*450*	5	2	UU				4	1	Up-regulated	2,76E-02	PMID:19380173	PMID:19662092		
PHB	*PHB*	*450*	5	2	UU				4	1	Up-regulated	2,76E-02	PMID:19380173	PMID:19662092		
CCT6A	*CCT6A*	*450*	5	2	UU				4	1	Up-regulated	2,76E-02	PMID:11034307	PMID:14732480		
DKK3	*DKK3*	*450*	4	1	D				4	1	Down-regulated	2,76E-02	PMID:15920167			
ACTA2	*ACTA2*	*450*	4	1	U				4	1	Up-regulated	2,76E-02	PMID:12912960			
CDC25A	*CDC25A*	*450*	5	2	UU				4	1	Up-regulated	2,76E-02	PMID:19380173	PMID:19662092		
RBBP4	*RBBP4*	*589*	5	1	D				5	1	Down-regulated	3,43E-02	PMID:12912960			
RNASE1	*RNASE1*	*589*	5	1	D				5	1	Down-regulated	3,43E-02	PMID:15920167			
RNF10	*RNF10*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:15920167			
SPINT2	*SPINT2*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:14732480			
ANXA11	*ANXA11*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:12912960			
SLC9A3R1	*SLC9A3R1*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:14732480			
DAB2	*DAB2*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:14732480			
DDR1	*DDR1*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:15920167			
TRIM29	*TRIM29*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:14732480			
CRYAB	*CRYAB*	*589*	5	1	D				5	1	Down-regulated	3,43E-02	PMID:19753302			
NR5A2	*NR5A2*	*589*	5	1	D				5	1	Down-regulated	3,43E-02	PMID:19662092			
SELL	*SELL*	*589*	5	1	D				5	1	Down-regulated	3,43E-02	PMID:19753302			
TNF	*TNF*	*589*	5	1	D				5	1	Down-regulated	3,43E-02	PMID:19662092			
C3AR1	*C3AR1*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:14732480			
COL10A1	*COL10A1*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:14732480			
DPYD	*DPYD*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:11034307			
INPPL1	*INPPL1*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:19753302			
KRT4	*KRT4*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:11251971			
PODXL	*PODXL*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:14732480			
TPR	*TPR*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:19753302			
BRCA2	*BRCA2*	*589*	6	2	UU				5	1	Up-regulated	3,43E-02	PMID:19380173	PMID:19662092		
NR3C2	*NR3C2*	*589*	5	1	D				5	1	Down-regulated	3,43E-02	PMID:19662092			
CDC6	*CDC6*	*589*	6	2	UU				5	1	Up-regulated	3,43E-02	PMID:19380173	PMID:19662092		
IARS	*IARS*	*589*	6	2	UU				5	1	Up-regulated	3,43E-02	PMID:11034307	PMID:14732480		
ACTG2	*ACTG2*	*589*	5	1	D				5	1	Down-regulated	3,43E-02	PMID:20043850			
AGER	*AGER*	*589*	5	1	D				5	1	Down-regulated	3,43E-02	PMID:15920167			
AKR1C1	*AKR1C1*	*589*	5	1	D				5	1	Down-regulated	3,43E-02	PMID:15920167			
ANG	*ANG*	*589*	5	1	D				5	1	Down-regulated	3,43E-02	PMID:19662092			
AP3B2	*AP3B2*	*589*	5	1	D				5	1	Down-regulated	3,43E-02	PMID:12912960			
ARHGDIG	*ARHGDIG*	*589*	5	1	D				5	1	Down-regulated	3,43E-02	PMID:11251971			
ATP1B3	*ATP1B3*	*589*	5	1	D				5	1	Down-regulated	3,43E-02	PMID:15920167			
BAI3	*BAI3*	*589*	5	1	D				5	1	Down-regulated	3,43E-02	PMID:12912960			
BCL6	*BCL6*	*589*	5	1	D				5	1	Down-regulated	3,43E-02	PMID:15920167			

BTG2	*BTG2*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
C7	*C7*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
CAT	*CAT*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
CD24	*CD24*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
CES1	*CES1*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
CREM	*CREM*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
CUL3	*CUL3*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:12912960
DMBT1	*DMBT1*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
DMD	*DMD*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:19753302
DYRK3	*DYRK3*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:12912960
EDNRB	*EDNRB*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
ELF3	*ELF3*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
ENPP2	*ENPP2*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
FBLN2	*FBLN2*	*589*	5	1		D	D	D		5	1	Down-regulated	3,43E-02	PMID:15136399
FPR1	*FPR1*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
GMFG	*GMFG*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
HBB	*HBB*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
HBG2	*HBG2*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
HPGD	*HPGD*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
IL15	*IL15*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:19662092
ITGB3	*ITGb3*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:11251971
LDB2	*LDB2*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
LPL	*LPL*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
LTBP1	*LTBP1*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
MAP2K3	*MAP2K3*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
MAT2A	*MAT2A*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
MITF	*MITF*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:19753302
NFIB	*NFIB*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
PARD6A	*PARD6A*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:12912960
PRSS2	*PRSS2*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:12912960
PTGES	*PTGES*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:11251971
RPL14	*RPL14*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
RPS11	*RPS11*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
SCD	*SCD*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:15920167
TGFBR3	*TGFBR3*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:19753302
TLE4	*TLE4*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:12912960
TNFRSF10C	*TNFRSF10C*	*589*	5	1	D					5	1	Down-regulated	3,43E-02	PMID:12912960
ACTR1A	*ACTR1A*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:12912960
ANXA7	*ANXA7*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:12912960
AP2M1	*AP2M1*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
APOL1	*APOL*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:14732480
ARHGDIB	*ARHGDIB*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:11251971
ATP2A2	*ATP2A2*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
ATRX	*ATRX*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:19753302
BAX	*BAX*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:19753302
BCOR	*BCOR*	*589*	5	1		U				5	1	Up-regulated	3,43E-02	PMID:15136399
BGN	*BGN*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:19753302
BLNK	*BLNK*	*589*	5	1		U	U	U		5	1	Up-regulated	3,43E-02	PMID:15136399
BUB3	*BUB3*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
CADM1	*CADM1*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:19380173
CALB2	*CALB2*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
CALD1	*CALD1*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
CCT7	*CCT7*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:19380173
CD177	*CD177*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:12912960
CD74	*CD74*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:14732480
CDC25B	*CDC25B*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:11251971
CDC7	*CDC7*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:19380173
CLIC4	*CLIC4*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
CLTA	*CLTA*	*589*	5	1			U			5	1	Up-regulated	3,43E-02	PMID:15136399
COPA	*COPA*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
DGCR6	*DGCR6*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
DTYMK	*DTYMK*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:19662092
ENO1	*ENO1*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
FABP5	*FABP5*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:11034307
FANCA	*FANCA*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:19380173
FGF12	*FGF12*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:11251971
FHL2	*FHL2*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
FZD6	*FZD6*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
GTF2H2	*GTF2H2*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:19380173
HSPA1A	*HSPA1A*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:14732480
HSPA8	*HSPA8*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:14732480
IFI16	*IFI16*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:14732480
IFITM3	*IFITM3*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:14732480
IGFBP2	*IGFBP2*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
IGHG3	*IGHG3*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:14732480
IL1RAP	*IL1RAP*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:19662092
ING1	*ING1*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:11251971
JAG1	*JAG1*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:11251971
KIFC3	*KIFC3*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:19380173
KPNA2	*KPNA2*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
KRT19	*KRT19*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
LILRB5	*LILRB5*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:14732480
LMNB1	*LMNB1*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:19753302
MAP3K14	*MAP3K14*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:11251971
MAPK7	*MAPK7*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:11251971
MAPK8	*MAPK8*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:11251971
MATK	*MATK*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:19380173
MEIS2	*MEIS2*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
MEST	*MEST*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
MID2	*MID2*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:12912960
MRC2	*MRC2*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:14732480
MRE11A	*MRE11A*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:11034307
MVP	*MVP*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:12912960
NAB1	*NAB1*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:14732480
NCBP1	*NCBP1*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:14732480
NDUFB4	*NDUFB4*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
NDUFC1	*NDUFC1*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
NMB	*NMB*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:12912960
NONO	*NONO*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
NOP56	*NOP56*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:14732480
OXCT1	*OXCT1*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:11034307
PABPC1	*PABPC1*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
PAIP1	*PAIP1*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
PAK1	*PAK1*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:11251971
PAWR	*PAWR*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
PAX6	*PAX6*	*589*	5	1		U	U	U		5	1	Up-regulated	3,43E-02	PMID:15136399
PDGFC	*PDGFC*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
PFN2	*PFN2*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
PGAP2	*PGAP2*	*589*	5	1		U				5	1	Up-regulated	3,43E-02	PMID:15136399
PPP1R12A	*PPP1R12A*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
PSMA4	*PSMA4*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:19380173
PSMB2	*PSMB2*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:19380173
PSMC5	*PSMC5*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:19380173
PSMD2	*PSMD2*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:19380173
PTMA	*PTMA*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:12912960
PTTG1	*PTTG1*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:19662092
PTTG1IP	*PTTG1IP*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:12912960
RAD50	*RAD50*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:19380173
RGS16	*RGS16*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:14732480
RGS5	*RGS5*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:14732480
RPLP0	*RPLP0*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
SAT1	*SAT*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:14732480
SFRP4	*SFRP4*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:14732480
SKIL	*SKIL*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:14732480
SLC16A1	*SLC16A1*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
SLC25A1	*SLC25A1*	*589*	5	1		U	U			5	1	Up-regulated	3,43E-02	PMID:15136399
SLC25A6	*SLC25A6*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:15920167
SLC3A2	*SLC3A2*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:14732480
SPHK1	*SPHK1*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:14732480
SRP72	*SRP72*	*589*	5	1	U					5	1	Up-regulated	3,43E-02	PMID:11034307

ST14	*ST14*	*589*	5	1		U	U	U	5	1	Up-regulated	3,43E-02	PMID:15136399		
SYNE1	*SYNE1*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:12912960		
TFG	*TFG*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:15920167		
TIMM17A	*TIMM17A*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:11034307		
TNXB	*TNXB*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:14732480		
TSPAN3	*TSPAN-3*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:15920167		
TXNRD1	*TXNRD1*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:15920167		
VEGFA	*VEGFA*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:19662092		
XRCC5	*XRCC5*	*589*	5	1	U				5	1	Up-regulated	3,43E-02	PMID:11251971		
ZNHIT1	*ZNHIT1*	*589*	5	1		U	U		5	1	Up-regulated	3,43E-02	PMID:15136399		
RRM1	*RRM1*	*589*	6	2	UU				5	1	Up-regulated	3,43E-02	PMID:19380173	PMID:19662092	
TYMS	*TYMS*	*589*	6	2	UU				5	1	Up-regulated	3,43E-02	PMID:19380173	PMID:19662092	
ZWINT	*ZWINT*	*589*	6	2	UU				5	1	Up-regulated	3,43E-02	PMID:19380173	PMID:19662092	
FOXO3	*FOXO3A*	*589*	5	1	D				5	1	Down-regulated	3,43E-02	PMID:19753302		
FOXO1	*FOXO1A*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:15920167		
MMP15	*MMP15*	*766*	6	1		U			6	1	Up-regulated	4,08E-02	PMID:15136399		
MTRR	*MTRR*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480		
TARS	*TARS*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:11034307		
NTRK2	*NTRK2*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:19753302		
ARL1	*ARL1*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
E2F2	*E2F2*	*766*	5	2	UU				6	1	Up-regulated	4,08E-02	PMID:19380173	PMID:19662092	
WT1	*WT1*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:19753302		
TIMP3	*TIMP3*	*766*	7	2	UU				6	1	Up-regulated	4,08E-02	PMID:11034307	PMID:14732480	
CCND2	*CCND2*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:11251971		
KRAS	*KRAS*	*766*	7	2	UU				6	1	Up-regulated	4,08E-02	PMID:11034307	PMID:14732480	
THBS1	*THBS1*	*766*	7	2	UU				6	1	Up-regulated	4,08E-02	PMID:11034307	PMID:14732480	
TPM1	*TPM1*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:19753302		
ABCB1	*ABCB1*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:19662092		
APOD	*APOD*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:15920167		
BSG	*BSG*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:11034307		
BTG1	*BTG1*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:19753302		
CASP1	*CASP1*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:15920167		
CAV2	*CAV2*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:15920167		
CD36	*CD36*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:15920167		
CSRP2	*CSRP2*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:15920167		
DCN	*DCN*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:15920167		
DLC1	*DLC1*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:19662092		
DPT	*DPT*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:15920167		
FABP4	*FABP4*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:15920167		
FDX1	*FDX1*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:12912960		
HLA-DQA1	*HLA-DQA1*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:15920167		
ICAM2	*ICAM2*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:19753302		
IGFBP4	*IGFBP4*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:11251971		
IL11RA	*IL11RA*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:19662092		
ITGA7	*ITGA7*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:19753302		
LEPR	*LEPR*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:15920167		
MAOA	*MAOA*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:15920167		
MGP	*MGP*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:15920167		
NR4A3	*NR4A3*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:15920167		
PDE4B	*PDE4B*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:19753302		
PDE4D	*PDE4D*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:15920167		
PPAP2B	*PPAP2B*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:15920167		
PTGER4	*PTGER4*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:15920167		
RARA	*RARA*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:15920167		
RARB	*RARB*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:11251971		
RPS20	*RPS20*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:15920167		
SOD2	*SOD2*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:15920167		
UQCRFS1	*Uqcrfs1*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:12912960		
NR3C1	*NR3C1*	*766*	6	2	DD				6	2	Down-regulated	4,08E-02	PMID:19753302	PMID:19662092	
ACLY	*ACLY*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:11034307		
AHCY	*AHCY*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
ANXA1	*ANXA1*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:11034307		
ANXA3	*ANXA3*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
APBB2	*APBB2*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:19753302		
APOE	*APOE*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480		
ARNTL	*ARNTL*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:19662092		
C5	*C5*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480		
CA10	*CA10*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480		
CALR	*CALR*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:12912960		
CALU	*CALU*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
CANX	*CANX*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
CASP10	*CASP10*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:11251971		
CD59	*CD59*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:11034307		
CIT	*CIT*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:19662092		
COL3A1	*COL3A1*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:12912960		
COL6A2	*COL6A2*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:12912960		
COMP	*COMP*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480		
COPB1	*COPB*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
CSRP1	*CSRP1*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480		
CXADR	*CXADR*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
DDOST	*DDOST*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
DFNA5	*DFNA5*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
DHFR	*DHFR*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:19753302		
DIO2	*DIO2*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:11034307		
EFEMP1	*EFEMP1*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
EIF3A	*EIF3A*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:12912960		
FAP	*FAP*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480		
FKBP1A	*FKBP1A*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
FZD2	*FZD2*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:11251971		
GTF2B	*GTF2B*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480		
GTF3A	*GTF3A*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480		
HINT1	*HINT*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
IGFBP7	*IGFBP7*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480		
IGSF1	*IGSF1*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480		
IQGAP1	*IQGAP1*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
IRAK1	*IRAK1*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
KDELR2	*KDELR2*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
KIAA0101	*KIAA0101*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
KRT17	*KRT17*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480		
LOX	*LOX*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
MAL	*MAL*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480		
MDK	*MDK*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:19753302		
MFAP2	*MFAP2*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:12912960		
MMP11	*MMP11*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:19753302		
MYC	*MYC*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:11034307		
MYLK	*MYLK*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480		
NPAS2	*NPAS2*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:19662092		
OGT	*OGT*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
PLOD2	*PLOD2*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
PPIB	*PPIB*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
PPM1G	*PPM1G*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480		
PRKCI	*PRKCI*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
PSAP	*PSAP*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480		
PSMC1	*PSMC1*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:19380173		
PSMC2	*PSMC2*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:19380173		
PSMC3	*PSMC3*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:19380173		
PSMD1	*PSMD1*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:19380173		
PTGS1	*PTGS1*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480		
RAD23B	*RAD23B*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
RARRES2	*RARRES2*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480		
RBMS1	*RBMS1*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
RGS1	*RGS1*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
SDC2	*SDC2*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480		
SERPINF1	*SERPINF1*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480		
SETMAR	*SETMAR*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:19380173		
SNRPD2	*SNRPD2*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
SNRPF	*SNRPF*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480		
STIP1	*STIP1*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:11034307		
TM4SF1	*TM4SF1*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		
TPD52L1	*TPD52L1*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167		

TPI1	*TPI1*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167			
TRAF2	*TRAF2*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:11251971			
TXNL1	*TXNL1*	*766*	6	1		U	U	U	6	1	Up-regulated	4,08E-02	PMID:15136399			
UCHL1	*UCHL1*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167			
UCP2	*UCP2*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480			
UQCRC2	*UQCRC2*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:12912960			
VCAM1	*VCAM1*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480			
VLDLR	*VLDLR*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:15920167			
WASL	*WASL*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:19753302			
ZNF177	*ZNF177*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480			
ZNF238	*ZNF238*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:14732480			
NME1	*NME1*	*766*	7	3	UUU				6	2	Up-regulated	4,08E-02	PMID:19380173	PMID:19662092	PMID:11034307	
PRKCA	*PRKCA*	*766*	7	2	UU				6	1	Up-regulated	4,08E-02	PMID:19380173	PMID:19662092		
CCNE2	*CCNE2*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:19753302			
CDKN1A	*CDKN1A*	*766*	6	1	D				6	1	Down-regulated	4,08E-02	PMID:19753302			
ACTB	*ACTB*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:12912960			
CCNA2	*CCNA2*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:19753302			
CCND1	*CCND1*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:11251971			
CCND3	*CCND3*	*766*	6	1	U				6	1	Up-regulated	4,08E-02	PMID:11251971			
FGF7	*FGF7*	*901*	7	1	D				7	1	Down-regulated	4,73E-02	PMID:11251971			
TNFSF10	*TNFSF10*	*901*	7	1	D				7	1	Down-regulated	4,73E-02	PMID:15920167			
EGR1	*EGR1*	*901*	7	1	D				7	1	Down-regulated	4,73E-02	PMID:15920167			
FN1	*FN1*	*901*	7	1	D				7	1	Down-regulated	4,73E-02	PMID:11034307			
IL6	*IL6*	*901*	7	1	D				7	1	Down-regulated	4,73E-02	PMID:15920167			
MCL1	*MCL1*	*901*	7	1	D				7	1	Down-regulated	4,73E-02	PMID:15920167			
SH3BP5	*SH3BP5*	*901*	7	1	D				7	1	Down-regulated	4,73E-02	PMID:15920167			
ADSL	*ADSL*	*901*	7	1			U	U	7	1	Up-regulated	4,73E-02	PMID:15136399			
AKT1	*AKT1*	*901*	7	1	U				7	1	Up-regulated	4,73E-02	PMID:12912960			
ALDH6A1	*ALDH6A1*	*901*	7	1	U				7	1	Up-regulated	4,73E-02	PMID:12912960			
ANXA8	*ANXA8*	*901*	7	1	U				7	1	Up-regulated	4,73E-02	PMID:15920167			
APP	*APP*	*901*	7	1	U				7	1	Up-regulated	4,73E-02	PMID:19753302			
ARHGDIA	*ARHGDIA*	*901*	7	1	U				7	1	Up-regulated	4,73E-02	PMID:12912960			
DAP3	*DAP3*	*901*	7	1	U				7	1	Up-regulated	4,73E-02	PMID:15920167			
EFNA1	*EFNA1*	*901*	7	1	U				7	1	Up-regulated	4,73E-02	PMID:14732480			
EPHB2	*EPHB2*	*901*	7	1	U				7	1	Up-regulated	4,73E-02	PMID:19753302			
FGFR3	*FGFR3*	*901*	7	1	U				7	1	Up-regulated	4,73E-02	PMID:19753302			
ITGA6	*ITGA6*	*901*	7	1	U				7	1	Up-regulated	4,73E-02	PMID:11034307			
KRT8	*KRT8*	*901*	7	1	U				7	1	Up-regulated	4,73E-02	PMID:15920167			
LAMB1	*LAMB1*	*901*	7	1	U				7	1	Up-regulated	4,73E-02	PMID:12912960			
LAMC1	*LAMC1*	*901*	7	1	U				7	1	Up-regulated	4,73E-02	PMID:11034307			
MET	*MET*	*901*	7	1	U				7	1	Up-regulated	4,73E-02	PMID:15920167			
PSME3	*PSME3*	*901*	7	1	U				7	1	Up-regulated	4,73E-02	PMID:19380173			
SPTAN1	*SPTAN1*	*901*	7	1	U				7	1	Up-regulated	4,73E-02	PMID:15920167			
TGFBI	*TGFBI*	*901*	7	1	U				7	1	Up-regulated	4,73E-02	PMID:15920167			
VIM	*VIM*	*901*	7	1	U				7	1	Up-regulated	4,73E-02	PMID:12912960			
ATM	*ATM*	*901*	7	1	U				7	1	Up-regulated	4,73E-02	PMID:14732480			
TP53	*TP53*	*901*	7	1	U				7	1	Up-regulated	4,73E-02	PMID:12912960			
FLT3	*FLT3*	*901*	7	1	U				7	1	Up-regulated	4,73E-02	PMID:11251971			
HMMR	*HMMR*	*930*	8	1	U				8	1	Up-regulated	5,37E-02	PMID:19753302			
KRT13	*KRT13*	*930*	8	1	U				8	1	Up-regulated	5,37E-02	PMID:14732480			
	Genes with too little evidences of de-regulation															
A2M	*A2M*		7	2	UD				7	2	Contradictory		PMID:14732480	PMID:15920167		
AARS	*AARS*		6	2	UD				6	2	Contradictory		PMID:19380173	PMID:12912960		
ALCAM	*ALCAM*		1	1		D	U	U	1	1	Inc. among histotypes		PMID:15136399			
BCL2	*BCL2*		7	2	UD				7	2	Contradictory		PMID:12912960	PMID:19662092		
BLVRB	*BLVRB*		4	1		U	U	D	4	1	Inc. among histotypes		PMID:15136399			
CALM2	*CALM2*		7	1			U	D	7	1	Inc. among histotypes		PMID:15136399			
CD9	*CD9*		7	1		U		D	7	1	Inc. among histotypes		PMID:15136399			
CXCL12	*CXCL12*		4	2	UD				4	2	Contradictory		PMID:14732480	PMID:15920167		
CYTH4	*CYT4*		1	1		U		D	1	1	Inc. among histotypes		PMID:15136399			
FCGR3B	*FCGR3B*		5	2	UD				5	2	Contradictory		PMID:14732480	PMID:15920167		
THNSL1	*FLJ22002*		1	1		U	U	D	1	1	Inc. among histotypes		PMID:15136399			
FOS	*FOS*		7	2	UD				7	2	Contradictory		PMID:14732480	PMID:19662092		
GABRB1	*GABRB1*		3	1		U	U	D	3	1	Inc. among histotypes		PMID:15136399			
GSN	*GSN*		7	3	UDD				7	3	Contradictory		PMID:14732480	PMID:19662092	PMID:15920167	
HLA-DRA	*HLA-DRA*		2	2	UD				2	2	Contradictory		PMID:14732480	PMID:15920167		
ICAM1	*ICAM1*		6	2	UD				6	2	Contradictory		PMID:14732480	PMID:15920167		
ID2	*ID2*		6	3	UUD				6	3	Contradictory		PMID:19753302	PMID:14732480	PMID:15920167	
IFNAR2	*IFNAR2*		5	1		U		D	5	1	Inc. among histotypes		PMID:15136399			
IGFBP5	*IGFBP5*		8	2	U	D	D	D	8	2	Inc. among histotypes		PMID:15136399	PMID:14732480		
LAMA4	*LAMA4*		6	2	UD				6	2	Contradictory		PMID:19753302	PMID:11034307		
LY6E	*LY6E*		3	1		U	D	D	3	1	Inc. among histotypes		PMID:15136399			
MCAM	*MCAM*		4	1	UD				4	1	Contradictory		PMID:15920167			
MIF	*MIF*		3	2	UD				3	2	Contradictory		PMID:11034307	PMID:15920167		
MRC1	*MRC1*		3	2	UD				3	2	Contradictory		PMID:14732480	PMID:15920167		
NR1D2	*NR1D2*		6	2	D		U	U	6	2	Inc. among histotypes		PMID:15136399	PMID:19662092		
NR4A1	*NR4A1*		6	3	UDD				6	3	Contradictory		PMID:14732480	PMID:15920167	PMID:19662092	
CXXC1	*PCCX1*		1	1		U		D	1	1	Inc. among histotypes		PMID:15136399			
PDZK1	*PDZK1*		4	1		U	U	D	4	1	Inc. among histotypes		PMID:15136399			
RB1	*RB1*		7	3	UUD				6	2	Contradictory		PMID:19380173	PMID:12912960	PMID:19662092	
RGS2	*RGS2*		5	2	UD				5	2	Contradictory		PMID:14732480	PMID:15920167		
S100A4	*S100A4*		5	2	UD				5	2	Contradictory		PMID:14732480	PMID:15920167		
SEPP1	*SEPP1*		6	2	UD				6	2	Contradictory		PMID:14732480	PMID:15920167		
SHC1	*SHC1*		5	1			U	D	5	1	Inc. among histotypes		PMID:15136399			
SRGN	*SRGN*		1	1		D	U		1	1	Inc. among histotypes		PMID:15136399			
TBL1X	*TBL1X*		5	1		D	U	D	5	1	Inc. among histotypes		PMID:15136399			
TGFB3	*TGFB3*		3	2	UD				3	2	Contradictory		PMID:11251971	PMID:14732480		
TSPAN15	*TSPAN15*		2	1		U	D	D	2	1	Inc. among histotypes		PMID:15136399			

BIBLIOGRAPHY

Alizadeh,A.A., M.B.Eisen, R.E.Davis, C.Ma, I.S.Lossos, A.Rosenwald, J.C.Boldrick, H.Sabet, T.Tran, X.Yu, J.I.Powell, L.Yang, G.E.Marti, T.Moore, J.Hudson, Jr., L.Lu, D.B.Lewis, R.Tibshirani, G.Sherlock, W.C.Chan, T.C.Greiner, D.D.Weisenburger, J.O.Armitage, R.Warnke, R.Levy, W.Wilson, M.R.Grever, J.C.Byrd, D.Botstein, P.O.Brown, and L.M.Staudt. 2000. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 403: 503-511.

Al-Taei S., J.Salimu, J.F.Lester, S.Linnane, M.Goonewardena, R.Harrop, M.D.Mason, and Z.Tabi. 2012. Overexpression and potential targeting of the oncofoetal antigen 5T4 in malignant pleural mesothelioma. Lung Cancer 77(2):312-8.

Amati,M., M.Tomasetti, M.Scartozzi, L.Mariotti, M.Ciuccarelli, M.Valentino, M.Governa, and L.Santarelli. 2007. [Biomarkers for prevention and early diagnosis of malignant pleural mesothelioma]. G. Ital. Med. Lav. Ergon. 29: 335-338.

Aoe,K., A.Hiraki, T.Murakami, S.Toyooka, N.Shivapurkar, A.F.Gazdar, N.Sueoka, K.Taguchi, T.Kamei, H.Takeyama, K.Sugi, and T.Kishimoto. 2006. Infrequent existence of simian virus 40 large T antigen DNA in malignant mesothelioma in Japan. Cancer Sci. 97: 292-295.

Armato,S.G., III, J.Entwisle, M.T.Truong, A.K.Nowak, G.L.Ceresoli, B.Zhao, R.Misri, and H.L.Kindler. 2008. Current state and future directions of pleural mesothelioma imaging. Lung Cancer 59: 411-420.

Ascoli,V., C.C.Scalzo, F.Facciolo, and F.Nardi. 1995. Platelet-derived growth factor receptor immunoreactivity in mesothelioma and nonneoplastic mesothelial cells in serous effusions. Acta Cytol. 39: 613-622.

Azim,H.A., Jr., R.Gaafar, S.Abdel, I, S.El-Guindy, I.Elattar, A.Ashmawy, and O.Khorshid. 2008. Soluble mesothelin-related protein in malignant pleural mesothelioma. J. Egypt. Natl. Canc. Inst. 20: 224-229.

Baselga,J. 2006. Targeting tyrosine kinases in cancer: the second wave. Science 312: 1175-1178.

Baser,M.E., R.A.De, D.Altomare, B.R.Balsara, N.M.Hedrick, D.H.Gutmann, L.H.Pitts, R.K.Jackler, and J.R.Testa. 2002. Neurofibromatosis 2 and malignant mesothelioma. Neurology 59: 290-291.

Benard,F., D.Sterman, R.J.Smith, L.R.Kaiser, S.M.Albelda, and A.Alavi. 1998. Metabolic imaging of malignant pleural mesothelioma with fluorodeoxyglucose positron emission tomography. Chest 114: 713-722.

Betsholtz,C., A.Johnsson, C.H.Heldin, B.Westermark, P.Lind, M.S.Urdea, R.Eddy, T.B.Shows, K.Philpott, A.L.Mellor, and . 1986. cDNA sequence and chromosomal localization of human platelet-derived growth factor A-chain and its expression in tumour cell lines. Nature 320: 695-699.

Betti,M., D.Ferrante, M.Padoan, S.Guarrera, M.Giordano, A.Aspesi, D.Mirabelli, C.Casadio, F.Ardissone, E.Ruffini, P.G.Betta, R.Libener, R.Guaschino, G.Matullo, E.Piccolini, C.Magnani, and I.Dianzani. 2011. XRCC1 and ERCC1 variants modify malignant mesothelioma risk: a case-control study. Mutat. Res. 708: 11-20.

Beyer,H.L., R.D.Geschwindt, C.L.Glover, L.Tran, I.Hellstrom, K.E.Hellstrom, M.C.Miller, T.Verch, W.J.Allard, H.I.Pass, and N.Y.Sardesai. 2007. MESOMARK: a potential test for malignant pleural mesothelioma. Clin. Chem. 53: 666-672.

Bharadwaj,U., C.Marin-Muller, M.Li, C.Chen, and Q.Yao. 2011a. Mesothelin confers pancreatic cancer cell resistance to TNF-alpha-induced apoptosis through Akt/PI3K/NF-kappaB activation and IL-6/Mcl-1 overexpression. Mol. Cancer 10: 106.

Bharadwaj,U., C.Marin-Muller, M.Li, C.Chen, and Q.Yao. 2011b. Mesothelin overexpression promotes autocrine IL-6/sIL-6R trans-signaling to stimulate pancreatic cancer cell proliferation. Carcinogenesis 32: 1013-1024.

Bianchi,A.B., S.I.Mitsunaga, J.Q.Cheng, W.M.Klein, S.C.Jhanwar, B.Seizinger, N.Kley, A.J.Klein-Szanto, and J.R.Testa. 1995. High frequency of inactivating mutations in the neurofibromatosis type 2 gene (NF2) in primary malignant mesotheliomas. Proc. Natl. Acad. Sci. U. S. A 92: 10854-10858.

Bohmer,F.D., L.Karagyozov, A.Uecker, H.Serve, A.Botzki, S.Mahboobi, and S.Dove. 2003. A single amino acid exchange inverts susceptibility of related receptor tyrosine kinases for the ATP site inhibitor STI-571. J. Biol. Chem. 278: 5148-5155.

Bott,M., M.Brevet, B.S.Taylor, S.Shimizu, T.Ito, L.Wang, J.Creaney, R.A.Lake, M.F.Zakowski, B.Reva, C.Sander, R.Delsite, S.Powell, Q.Zhou, R.Shen, A.Olshen, V.Rusch, and M.Ladanyi. 2011. The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. Nat. Genet. 43: 668-672.

Boutin,C. and F.Rey. 1993. Thoracoscopy in pleural malignant mesothelioma: a prospective study of 188 consecutive patients. Part 1: Diagnosis. Cancer 72: 389-393.

Brase J.C., M.Schmidt, T.Fischbach, H.Sültmann, H.Bojar, H.Koelbl, B.Hellwig, J.Rahnenführer, J.G.Hengstler, and M.C.Gehrmann. 2010. ERBB2 and TOP2A in breast cancer: a comprehensive analysis of gene amplification, RNA levels, and protein expression and their influence on prognosis and prediction. Clin Cancer Res 16:2391-401.

Brozic P., J.Cesar, A.Kovac, M.Davies, A.P.Johnson, C.W.Fishwick, T.Lanisnik Rizner, and S.Gobec. 2009. Derivatives of pyrimidine, phthalimide and anthranilic acid as inhibitors of human hydroxysteroid dehydrogenase AKR1C1. Chem Biol Interact 16;178(1-3):158-64.

Bustin,S.A., V.Benes, J.A.Garson, J.Hellemans, J.Huggett, M.Kubista, R.Mueller, T.Nolan, M.W.Pfaffl, G.L.Shipley, J.Vandesompele, and C.T.Wittwer. 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin. Chem. 55: 611-622.

Cacciotti,P., R.Libener, P.Betta, F.Martini, C.Porta, A.Procopio, L.Strizzi, L.Penengo, M.Tognon, L.Mutti, and G.Gaudino. 2001. SV40 replication in human mesothelial cells induces HGF/Met receptor activation: a model for viral-related carcinogenesis of human malignant mesothelioma. Proc. Natl. Acad. Sci. U. S. A 98: 12032-12037.

Camp,A.J. and R.Wijesinghe. 2009. Calretinin: modulator of neuronal excitability. Int. J. Biochem. Cell Biol. 41: 2118-2121.

Cao,X., C.Rodarte, L.Zhang, C.D.Morgan, J.Littlejohn, and W.R.Smythe. 2007. Bcl2/bcl-xL inhibitor engenders apoptosis and increases chemosensitivity in mesothelioma. Cancer Biol. Ther. 6: 246-252.

Carbone,M. and C.W.Bedrossian. 2006. The pathogenesis of mesothelioma. Semin. Diagn. Pathol. 23: 56-60.

Carbone,M., S.Emri, A.U.Dogan, I.Steele, M.Tuncer, H.I.Pass, and Y.I.Baris. 2007. A mesothelioma epidemic in Cappadocia: scientific developments and unexpected social outcomes. Nat. Rev. Cancer 7: 147-154.

Carbone,M., L.K.Ferris, F.Baumann, A.Napolitano, C.A.Lum, E.G.Flores, G.Gaudino, A.Powers, P.Bryant-Greenwood, T.Krausz, E.Hyjek, R.Tate, J.Friedberg, T.Weigel, H.I.Pass, and H.Yang. 2012. BAP1 cancer syndrome: malignant mesothelioma, uveal and cutaneous melanoma, and MBAITs. J. Transl. Med. 10: 179.

Carbone,M., R.A.Kratzke, and J.R.Testa. 2002. The pathogenesis of mesothelioma. Semin. Oncol. 29: 2-17.

Carbone,M., P.Rizzo, P.M.Grimley, A.Procopio, D.J.Mew, V.Shridhar, B.A.de, V.Esposito, M.T.Giuliano, S.M.Steinberg, A.S.Levine, A.Giordano, and H.I.Pass. 1997. Simian virus-40 large-T antigen binds p53 in human mesotheliomas. Nat. Med. 3: 908-912.

Carthew,R.W. and E.J.Sontheimer. 2009. Origins and Mechanisms of miRNAs and siRNAs. Cell 136: 642-655.

Chang,K. and I.Pastan. 1996. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. Proc. Natl. Acad. Sci. U. S. A 93: 136-140.

Cheung,M., J.Pei, Y.Pei, S.C.Jhanwar, H.I.Pass, and J.R.Testa. 2010. The promyelocytic leukemia zinc-finger gene, PLZF, is frequently downregulated in malignant mesothelioma cells and contributes to cell survival. Oncogene 29: 1633-1640.

Chiosea,S., A.Krasinskas, P.T.Cagle, K.A.Mitchell, D.S.Zander, and S.Dacic. 2008. Diagnostic importance of 9p21 homozygous deletion in malignant mesotheliomas. Mod. Pathol. 21: 742-747.

Chon,H.S. and J.M.Lancaster. 2011. Microarray-based gene expression studies in ovarian cancer. Cancer Control 18: 8-15.

Chowdhury P.S., and I.Pastan. 1999. Improving antibody affinity by mimicking somatic hypermutation in vitro. Nat Biotechnol 17(6):568-72.

Chowdhury P.S., J.L.Viner, R.Beers, and I.Pastan. 1998. Isolation of a high-affinity stable single-chain Fv specific for mesothelin from DNA-immunized mice by phage display and construction of a recombinant immunotoxin with anti-tumor activity. Proc Natl Acad Sci U S A. 95(2):669-74.

Chresta,C.M., J.R.Masters, and J.A.Hickman. 1996. Hypersensitivity of human testicular tumors to etoposide-induced apoptosis is associated with functional p53 and a high Bax:Bcl-2 ratio. Cancer Res. 56: 1834-1841.

Christensen,B.C., E.A.Houseman, G.M.Poage, J.J.Godleski, R.Bueno, D.J.Sugarbaker, J.K.Wiencke, H.H.Nelson, C.J.Marsit, and K.T.Kelsey. 2010. Integrated profiling reveals a global correlation between epigenetic and genetic alterations in mesothelioma. Cancer Res. 70: 5686-5694.

Claesson-Welsh,L., A.Eriksson, A.Moren, L.Severinsson, B.Ek, A.Ostman, C.Betsholtz, and C.H.Heldin. 1988. cDNA cloning and expression of a human platelet-derived growth factor (PDGF) receptor specific for B-chain-containing PDGF molecules. Mol. Cell Biol. 8: 3476-3486.

Corallini,A., E.Mazzoni, A.Taronna, M.Manfrini, G.Carandina, G.Guerra, R.Guaschino, F.Vaniglia, C.Magnani, F.Casali, R.Dolcetti, C.Palmonari, G.Rezza, F.Martini, G.Barbanti-Brodano, and M.G.Tognon. 2012. Specific antibodies reacting with simian virus 40 capsid protein mimotopes in serum samples from healthy blood donors. Hum. Immunol. 73: 502-510.

Corless,C.L., A.Schroeder, D.Griffith, A.Town, L.McGreevey, P.Harrell, S.Shiraga, T.Bainbridge, J.Morich, and M.C.Heinrich. 2005. PDGFRA mutations in gastrointestinal stromal tumors: frequency, spectrum and in vitro sensitivity to imatinib. J. Clin. Oncol. 23: 5357-5364.

Creaney,J., R.J.Francis, I.M.Dick, A.W.Musk, B.W.Robinson, M.J.Byrne, and A.K.Nowak. 2011. Serum soluble mesothelin concentrations in malignant pleural mesothelioma: relationship to tumor volume, clinical stage and changes in tumor burden. Clin. Cancer Res. 17: 1181-1189.

Creaney,J., B.van, I, M.Hof, A.Segal, A.W.Musk, K.N.de, N.Horick, S.J.Skates, and B.W.Robinson. 2007. Combined CA125 and mesothelin levels for the diagnosis of malignant mesothelioma. Chest 132: 1239-1246.

Creaney,J., D.Yeoman, Y.Demelker, A.Segal, A.W.Musk, S.J.Skates, and B.W.Robinson. 2008. Comparison of osteopontin, megakaryocyte potentiating factor, and mesothelin proteins as markers in the serum of patients with malignant mesothelioma. J. Thorac. Oncol. 3: 851-857.

Creaney,J., D.Yeoman, L.K.Naumoff, M.Hof, A.Segal, A.W.Musk, K.N.de, N.Horick, S.J.Skates, and B.W.Robinson. 2007b. Soluble mesothelin in effusions: a useful tool for the diagnosis of malignant mesothelioma. Thorax 62: 569-576.

Crispi,S., R.A.Calogero, M.Santini, P.Mellone, B.Vincenzi, G.Citro, G.Vicidomini, S.Fasano, R.Meccariello, G.Cobellis, S.Menegozzo, R.Pierantoni, F.Facciolo, A.Baldi, and M.Menegozzo. 2009. Global gene expression profiling of human pleural mesotheliomas: identification of matrix metalloproteinase 14 (MMP-14) as potential tumour target. PLoS. One. 4: e7016.

Cristaudo,A., A.Bonotti, S.Simonini, R.Bruno, and R.Foddis. 2011a. Soluble markers for diagnosis of malignant pleural mesothelioma. Biomark. Med. 5: 261-273.

Cristaudo,A., A.Bonotti, S.Simonini, A.Vivaldi, G.Guglielmi, N.Ambrosino, A.Chella, M.Lucchi, A.Mussi, and R.Foddis. 2011b. Combined serum mesothelin and plasma osteopontin measurements in malignant pleural mesothelioma. J. Thorac. Oncol. 6: 1587-1593.

Cristaudo,A., R.Foddis, A.Vivaldi, R.Buselli, V.Gattini, G.Guglielmi, F.Cosentino, F.Ottenga, E.Ciancia, R.Libener, R.Filiberti, M.Neri, P.Betta, M.Tognon, L.Mutti, and R.Puntoni. 2005. SV40 enhances the risk of malignant mesothelioma among people exposed to asbestos: a molecular epidemiologic case-control study. Cancer Res. 65: 3049-3052.

Cristaudo,A., R.Foddis, A.Vivaldi, G.Guglielmi, N.Dipalma, R.Filiberti, M.Neri, M.Ceppi, M.Paganuzzi, G.P.Ivaldi, M.Mencoboni, P.A.Canessa, N.Ambrosino, A.Chella, L.Mutti, and R.Puntoni. 2007. Clinical significance of serum mesothelin in patients with mesothelioma and lung cancer. Clin. Cancer Res. 13: 5076-5081.

Dainty,L.A., J.I.Risinger, C.Morrison, G.V.Chandramouli, M.A.Bidus, C.Zahn, G.S.Rose, J.Fowler, A.Berchuck, and G.L.Maxwell. 2007. Overexpression of folate binding protein and mesothelin are associated with uterine serous carcinoma. Gynecol. Oncol. 105: 563-570.

Danial,N.N. and S.J.Korsmeyer. 2004. Cell death: critical control points. Cell 116: 205-219.

Davies,H.E., R.S.Sadler, S.Bielsa, N.A.Maskell, N.M.Rahman, R.J.Davies, B.L.Ferry, and Y.C.Lee. 2009. Clinical impact and reliability of pleural fluid mesothelin in undiagnosed pleural effusions. Am. J. Respir. Crit Care Med. 180: 437-444.

Deguen,B., L.Goutebroze, M.Giovannini, C.Boisson, N.R.van der, M.C.Jaurand, and G.Thomas. 1998. Heterogeneity of mesothelioma cell lines as defined by altered genomic structure and expression of the NF2 gene. Int. J. Cancer 77: 554-560.

Delgermaa,V., K.Takahashi, E.K.Park, G.V.Le, T.Hara, and T.Sorahan. 2011a. Global mesothelioma deaths reported to the World Health Organization between 1994 and 2008. Bull. World Health Organ 89: 716-724C.

Delgermaa,V., K.Takahashi, E.K.Park, G.V.Le, T.Hara, and T.Sorahan. 2011b. Global mesothelioma deaths reported to the World Health Organization between 1994 and 2008. Bull. World Health Organ 89: 716-724C.

Demirag,F., E.Unsal, A.Yilmaz, and A.Caglar. 2005. Prognostic significance of vascular endothelial growth factor, tumor necrosis, and mitotic activity index in malignant pleural mesothelioma. Chest 128: 3382-3387.

Dianzani,I., L.Gibello, A.Biava, M.Giordano, M.Bertolotti, M.Betti, D.Ferrante, S.Guarrera, G.P.Betta, D.Mirabelli, G.Matullo, and C.Magnani. 2006. Polymorphisms in DNA repair genes as risk factors for asbestos-related malignant mesothelioma in a general population study. Mutat. Res. 599: 124-134.

Dinu,M., R.N.Ciurea, M.Stefan, and A.C.Georgescu. 2012. The role of immunohistochemistry in the diagnosis of neoplastic pleural effusions. Rom. J. Morphol. Embryol. 53: 817-820.

Donaldson,K., F.A.Murphy, R.Duffin, and C.A.Poland. 2010. Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma. Part Fibre. Toxicol. 7: 5.

Dong,L., R.V.Jensen, R.A.De, G.J.Gordon, Y.Xu, D.J.Sugarbaker, and R.Bueno. 2009. Differentially expressed alternatively spliced genes in malignant pleural mesothelioma identified using massively parallel transcriptome sequencing. BMC. Med. Genet. 10: 149.

Druker,B.J. 2006. Circumventing resistance to kinase-inhibitor therapy. N. Engl. J. Med. 354: 2594-2596.

Dykxhoorn,D.M. 2009. RNA interference as an anticancer therapy: a patent perspective. Expert. Opin. Ther. Pat 19: 475-491.

EDDY,B.E. 1964. SIMIAN VIRUS 40 (SV-40): AN ONCOGENIC VIRUS. Prog. Exp. Tumor Res. 4: 1-26.

Elbashir,S.M., J.Harborth, W.Lendeckel, A.Yalcin, K.Weber, and T.Tuschl. 2001. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 411: 494-498.

Fennell,D.A. 2011. Genetics and molecular biology of mesothelioma. Recent Results Cancer Res. 189: 149-167.

Fitzpatrick,D.R., H.Bielefeldt-Ohmann, R.P.Himbeck, A.G.Jarnicki, A.L.Marzo, and B.W.Robinson. 1994. Transforming growth factor-beta: antisense RNA-mediated inhibition affects anchorage-independent growth, tumorigenicity and tumor-infiltrating T-cells in malignant mesothelioma. Growth Factors 11: 29-44.

Flejter,W.L., F.P.Li, K.H.Antman, and J.R.Testa. 1989. Recurring loss involving chromosomes 1, 3, and 22 in malignant mesothelioma: possible sites of tumor suppressor genes. Genes Chromosomes. Cancer 1: 148-154.

Fleury-Feith,J., C.Lecomte, A.Renier, M.Matrat, L.Kheuang, V.Abramowski, F.Levy, A.Janin, M.Giovannini, and M.C.Jaurand. 2003. Hemizygosity of Nf2 is associated with increased susceptibility to asbestos-induced peritoneal tumours. Oncogene 22: 3799-3805.

Fox,S. and A.Dharmarajan. 2006. WNT signaling in malignant mesothelioma. Front Biosci. 11: 2106-2112.

Freeman A., L.S.Morris, A.D.Mills, K.Stoeber, R.A.Laskey, G.H.Williams, and N.Coleman. 1999. Minichromosome maintenance proteins as biological markers of dysplasia and malignancy. *Clin* Cancer Res 5:2121-32.

Gander J.C., V.Gotzos, B.Fellay, and B.Schwaller. 1996. Inhibition of the proliferative cycle and apoptotic events in WiDr cells after down-regulation of the calcium-binding protein calretinin using antisense oligodeoxynucleotides. Exp Cell Res 15;225(2):399-410.

Gavrilov,K. and W.M.Saltzman. 2012. Therapeutic siRNA: principles, challenges, and strategies. Yale J. Biol. Med. 85: 187-200.

Gazdar,A.F., J.S.Butel, and M.Carbone. 2002. SV40 and human tumours: myth, association or causality? Nat. Rev. Cancer 2: 957-964.

Gerwin,B.I., J.F.Lechner, R.R.Reddel, A.B.Roberts, K.C.Robbins, E.W.Gabrielson, and C.C.Harris. 1987. Comparison of production of transforming growth factor-beta and platelet-derived growth factor by normal human mesothelial cells and mesothelioma cell lines. Cancer Res. 47: 6180-6184.

Gil,J. and G.Peters. 2006. Regulation of the INK4b-ARF-INK4a tumour suppressor locus: all for one or one for all. Nat. Rev. Mol. Cell Biol. 7: 667-677.

Godley,L.A., J.Cunningham, M.E.Dolan, R.S.Huang, S.Gurbuxani, M.E.McNerney, R.A.Larson, H.Leong, Y.Lussier, K.Onel, O.Odenike, W.Stock, K.P.White, and M.M.Le Beau. 2011. An integrated genomic approach to the assessment and treatment of acute myeloid leukemia. Semin. Oncol. 38: 215-224.

Gordon,G.J., K.Appasani, J.P.Parcells, N.K.Mukhopadhyay, M.T.Jaklitsch, W.G.Richards, D.J.Sugarbaker, and R.Bueno. 2002. Inhibitor of apoptosis protein-1 promotes tumor cell survival in mesothelioma. Carcinogenesis 23: 1017-1024.

Gordon,G.J., R.V.Jensen, L.L.Hsiao, S.R.Gullans, J.E.Blumenstock, W.G.Richards, M.T.Jaklitsch, D.J.Sugarbaker, and R.Bueno. 2003. Using gene expression ratios to predict outcome among patients with mesothelioma. J. Natl. Cancer Inst. 95: 598-605.

Gordon,G.J., G.N.Rockwell, R.V.Jensen, J.G.Rheinwald, J.N.Glickman, J.P.Aronson, B.J.Pottorf, M.D.Nitz, W.G.Richards, D.J.Sugarbaker, and R.Bueno. 2005. Identification of novel candidate oncogenes and tumor suppressors in malignant pleural mesothelioma using large-scale transcriptional profiling. Am. J. Pathol. 166: 1827-1840.

Goto,Y., K.Shinjo, Y.Kondo, L.Shen, M.Toyota, H.Suzuki, W.Gao, B.An, M.Fujii, H.Murakami, H.Osada, T.Taniguchi, N.Usami, M.Kondo, Y.Hasegawa, K.Shimokata, K.Matsuo, T.Hida, N.Fujimoto, T.Kishimoto, J.P.Issa, and Y.Sekido. 2009. Epigenetic profiles distinguish malignant pleural mesothelioma from lung adenocarcinoma. Cancer Res. 69: 9073-9082.

Grigoriu,B.D., B.Chahine, A.Vachani, T.Gey, M.Conti, D.H.Sterman, G.Marchandise, H.Porte, S.M.Albelda, and A.Scherpereel. 2009. Kinetics of soluble mesothelin in patients with malignant pleural mesothelioma during treatment. Am. J. Respir. Crit Care Med. 179: 950-954.

Grigoriu,B.D., A.Scherpereel, P.Devos, B.Chahine, M.Letourneux, P.Lebailly, M.Gregoire, H.Porte, M.C.Copin, and P.Lassalle. 2007. Utility of osteopontin and serum mesothelin in malignant pleural mesothelioma diagnosis and prognosis assessment. Clin. Cancer Res. 13: 2928-2935.

Hamilton,R.F., Jr., S.A.Thakur, and A.Holian. 2008. Silica binding and toxicity in alveolar macrophages. Free Radic. Biol. Med. 44: 1246-1258.

Hammacher,A., U.Hellman, A.Johnsson, A.Ostman, K.Gunnarsson, B.Westermark, A.Wasteson, and C.H.Heldin. 1988. A major part of platelet-derived growth factor purified from human platelets is a heterodimer of one A and one B chain. J. Biol. Chem. 263: 16493-16498.

Hansen,J., N.H.de Klerk, A.W.Musk, and M.S.Hobbs. 1998. Environmental exposure to crocidolite and mesothelioma: exposure-response relationships. Am. J. Respir. Crit Care Med. 157: 69-75.

Hansteen,I.L., B.Hilt, J.T.Lien, V.Skaug, and A.Haugen. 1993. Karyotypic changes in the preclinical and subsequent stages of malignant mesothelioma: a case report. Cancer Genet. Cytogenet. 70: 94-98.

Hart,C.E., J.W.Forstrom, J.D.Kelly, R.A.Seifert, R.A.Smith, R.Ross, M.J.Murray, and D.F.Bowen-Pope. 1988. Two classes of PDGF receptor recognize different isoforms of PDGF. Science 240: 1529-1531.

Hassan,R., T.Bera, and I.Pastan. 2004. Mesothelin: a new target for immunotherapy. Clin. Cancer Res. 10: 3937-3942.

Hassan,R., S.Bullock, A.Premkumar, R.J.Kreitman, H.Kindler, M.C.Willingham, and I.Pastan. 2007. Phase I study of SS1P, a recombinant anti-mesothelin immunotoxin given as a bolus I.V. infusion to patients with mesothelin-expressing mesothelioma, ovarian, and pancreatic cancers. Clin Cancer Res 13(17):5144-9.

Hassan R., S.J.Cohen, M.Phillips, I.Pastan, E.Sharon, R.J.Kelly, C.Schweizer, S.Weil, and D.Laheru. 2010. Phase I clinical trial of the chimeric anti-mesothelin monoclonal antibody MORAb-009 in patients with mesothelin-expressing cancers. Clin Cancer Res 16(24):6132-8.

Hassan R., W.Ebel, E.L.Routhier, R.Patel, J.B.Kline, J.Zhang, Q.Chao, S.Jacob, H.Turchin, L.Gibbs, M.D.Phillips, S.Mudali, C.Iacobuzio-Donahue, E.M.Jaffee, M.Moreno, I.Pastan, P.M.Sass, N.C.Nicolaides, and L.Grasso. 2007. Preclinical evaluation of MORAb-009, a chimeric antibody targeting tumor-associated mesothelin. Cancer Immun 19;7:20.

Hassan,R. and M.Ho. 2008. Mesothelin targeted cancer immunotherapy. Eur. J. Cancer 44: 46-53.

Hassan,R., A.T.Remaley, M.L.Sampson, J.Zhang, D.D.Cox, J.Pingpank, R.Alexander, M.Willingham, I.Pastan, and M.Onda. 2006. Detection and quantitation of serum mesothelin, a tumor marker for patients with mesothelioma and ovarian cancer. Clin. Cancer Res. 12: 447-453.

Haussecker,D. 2008. The business of RNAi therapeutics. Hum. Gene Ther. 19: 451-462.

Hayes,J.D., J.U.Flanagan, and I.R.Jowsey. 2005. Glutathione transferases. Annu. Rev. Pharmacol. Toxicol. 45: 51-88.

Heinrich,M.C., C.L.Corless, C.D.Blanke, G.D.Demetri, H.Joensuu, P.J.Roberts, B.L.Eisenberg, M.M.von, C.D.Fletcher, K.Sandau, K.McDougall, W.B.Ou, C.J.Chen, and J.A.Fletcher. 2006. Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. J. Clin. Oncol. 24: 4764-4774.

Heldin,C.H., A.Johnsson, S.Wennergren, C.Wernstedt, C.Betsholtz, and B.Westermark. 1986. A human osteosarcoma cell line secretes a growth factor structurally related to a homodimer of PDGF A-chains. Nature 319: 511-514.

Hellemans,J., G.Mortier, P.A.De, F.Speleman, and J.Vandesompele. 2007. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol. 8: R19.

Henzi,T., W.V.Blum, M.Pfefferli, T.J.Kawecki, V.Salicio, and B.Schwaller. 2009. SV40-induced expression of calretinin protects mesothelial cells from asbestos cytotoxicity and may be a key factor contributing to mesothelioma pathogenesis. Am. J. Pathol. 174: 2324-2336.

Hirvonen,A., K.Pelin, L.Tammilehto, A.Karjalainen, K.Mattson, and K.Linnainmaa. 1995. Inherited GSTM1 and NAT2 defects as concurrent risk modifiers in asbestos-related human malignant mesothelioma. Cancer Res. 55: 2981-2983.

Hirvonen,A., S.T.Saarikoski, K.Linnainmaa, K.Koskinen, K.Husgafvel-Pursiainen, K.Mattson, and H.Vainio. 1996. Glutathione S-transferase and N-acetyltransferase genotypes and asbestos-associated pulmonary disorders. J. Natl. Cancer Inst. 88: 1853-1856.

Ho,M., T.K.Bera, M.C.Willingham, M.Onda, R.Hassan, D.FitzGerald, and I.Pastan. 2007. Mesothelin expression in human lung cancer. Clin. Cancer Res. 13: 1571-1575.

Hoang,C.D., J.D'Cunha, M.G.Kratzke, C.E.Casmey, S.P.Frizelle, M.A.Maddaus, and R.A.Kratzke. 2004. Gene expression profiling identifies matriptase overexpression in malignant mesothelioma. Chest 125: 1843-1852.

Hodgson,J.T. and A.Darnton. 2000. The quantitative risks of mesothelioma and lung cancer in relation to asbestos exposure. Ann. Occup. Hyg. 44: 565-601.

Hollevoet,K., K.Nackaerts, J.Thimpont, P.Germonpre, L.Bosquee, V.P.de, C.Legrand, E.Kellen, Y.Kishi, J.R.Delanghe, and J.P.van Meerbeeck. 2010. Diagnostic performance of soluble mesothelin and megakaryocyte potentiating factor in mesothelioma. Am. J. Respir. Crit Care Med. 181: 620-625.

Hyun T.S., M.Barnes, and Z.L.Tabatabai. 2012. The diagnostic utility of D2-40, calretinin, CK5/6, desmin and MOC-31 in the differentiation of mesothelioma from adenocarcinoma in pleural effusion cytology. Acta Cytol 56(5):527-32.

Inase,N., S.Miyake, and Y.Yoshizawa. 2001. Calretinin promoter for suicide gene expression in malignant mesothelioma. Anticancer Res. 21: 1111-1114.

Ismail-Khan,R., L.A.Robinson, C.C.Williams, Jr., C.R.Garrett, G.Bepler, and G.R.Simon. 2006. Malignant pleural mesothelioma: a comprehensive review. Cancer Control 13: 255-263.

Ivanchuk,S.M., S.Mondal, P.B.Dirks, and J.T.Rutka. 2001. The INK4A/ARF locus: role in cell cycle control and apoptosis and implications for glioma growth. J. Neurooncol. 51: 219-229.

Ivanov,S.V., J.Miller, R.Lucito, C.Tang, A.V.Ivanova, J.Pei, M.Carbone, C.Cruz, A.Beck, C.Webb, D.Nonaka, J.R.Testa, and H.I.Pass. 2009. Genomic events associated with progression of pleural malignant mesothelioma. Int. J. Cancer 124: 589-599.

Iwahori,K., T.Osaki, S.Serada, M.Fujimoto, H.Suzuki, Y.Kishi, A.Yokoyama, H.Hamada, Y.Fujii, K.Yamaguchi, T.Hirashima, K.Matsui, I.Tachibana, Y.Nakamura, I.Kawase, and T.Naka. 2008. Megakaryocyte potentiating factor as a tumor marker of malignant pleural mesothelioma: evaluation in comparison with mesothelin. Lung Cancer 62: 45-54.

Jagadeeswaran,R., P.C.Ma, T.Y.Seiwert, S.Jagadeeswaran, O.Zumba, V.Nallasura, S.Ahmed, R.Filiberti, M.Paganuzzi, R.Puntoni, R.A.Kratzke, G.J.Gordon, D.J.Sugarbaker, R.Bueno, V.Janamanchi, V.P.Bindokas, H.L.Kindler, and R.Salgia. 2006. Functional analysis of c-Met/hepatocyte growth factor pathway in malignant pleural mesothelioma. Cancer Res. 66: 352-361.

Jain S., M.I.Filipe, P.A.Hall, N.Waseem, D.P.Lane, and D.A.Levison. 1991. Prognostic value of proliferating cell nuclear antigen in gastric carcinoma. J Clin Pathol 44:655-9.

Jean,D., E.Thomas, E.Manie, A.Renier, R.A.de, C.Lecomte, P.Andujar, J.Fleury-Feith, F.Galateau-Salle, M.Giovannini, J.Zucman-Rossi, M.H.Stern, and M.C.Jaurand. 2011. Syntenic relationships between genomic profiles of fibres-induced murine and human malignant mesothelioma. Am. J. Pathol. 178: 881-894.

Kao S.C., K.Griggs, K.Lee, N.Armstrong, S.Clarke, J.Vardy, N.van Zandwijk, J.Burn, B.C.McCaughan, D.W.Henderson, and S.Klebe. 2011. Validation of a minimal panel of antibodies for the diagnosis of malignant pleural mesothelioma. Pathology 43(4):313-7.

Kao,S.C., G.Reid, K.Lee, J.Vardy, S.Clarke, and Z.N.van. 2010. Malignant mesothelioma. Intern. Med. J. 40: 742-750.

Kelly R.J., E.Sharon, I.Pastan, and R.Hassan. 2012. Mesothelin-targeted agents in clinical trials and in preclinical development. Mol Cancer Ther 11(3):517-25.

Kettunen,E., A.M.Nissen, T.Ollikainen, M.Taavitsainen, J.Tapper, K.Mattson, K.Linnainmaa, S.Knuutila, and W.El-Rifai. 2001. Gene expression profiling of malignant mesothelioma cell lines: cDNA array study. Int. J. Cancer 91: 492-496.

Kim J.M., H.Y. Sohn, S.Y. Yoon, J.H. Oh, J.O. Yang, J.H. Kim, K.S. Song, S.M. Rho, H.S. Yoo, Y.S. Kim, J.G. Kim, and N.S. Kim. 2005. Identification of gastric cancer-related genes using a cDNA microarray containing novel expressed sequence tags expressed in gastric cancer cells. Clin Cancer Res 11:473-82.

Kinnula,V.L., P.Pietarinen-Runtti, K.Raivio, K.Kahlos, K.Pelin, K.Mattson, K.Linnainmaa. 1996. Manganese superoxide dismutase in human pleural mesothelioma cell lines. Free Radic Biol Med. 21(4):527-32.

Kinoshita Y., K.Takasu, T.Yuri, K.Yoshizawa, N.Uehara, A.Kimura, H.Miki, A.Tsubura, and N.Shikata. 2013. Two cases of malignant peritoneal mesothelioma without asbestos exposure: cytologic and immunohistochemical features. Ann Diagn Pathol 17(1):99-103.

Klein,G., A.Powers, and C.Croce. 2002. Association of SV40 with human tumors. Oncogene 21: 1141-1149.

Kleinberg,L., A.K.Lie, V.A.Florenes, J.M.Nesland, and B.Davidson. 2007. Expression of inhibitor-of-apoptosis protein family members in malignant mesothelioma. Hum. Pathol. 38: 986-994.

Knobel,P.A., I.N.Kotov, E.Felley-Bosco, R.A.Stahel, and T.M.Marti. 2011. Inhibition of REV3 expression induces persistent DNA damage and growth arrest in cancer cells. Neoplasia. 13: 961-970.

Krause A., V.Combaret, I.Iacono, B.Lacroix, C.Compagnon, C.Bergeron, S.Valsesia-Wittmann, P.Leissner, B.Mougin, and A.Puisieux. 2005. Genome-wide analysis of gene expression in neuroblastomas detected by mass screening. Cancer Lett 225:111-20.

Kreitman R.J., R.Hassan, D.J.Fitzgerald, and I.Pastan. 2009. Phase I trial of continuous infusion anti-mesothelin recombinant immunotoxin SS1P. Clin Cancer Res 15(16):5274-9.

Krismann,M., K.M.Muller, M.Jaworska, and G.Johnen. 2002. Molecular cytogenetic differences between histological subtypes of malignant mesotheliomas: DNA cytometry and comparative genomic hybridization of 90 cases. J. Pathol. 197: 363-371.

Kroczynska,B., R.Cutrone, M.Bocchetta, H.Yang, A.G.Elmishad, P.Vacek, M.Ramos-Nino, B.T.Mossman, H.I.Pass, and M.Carbone. 2006. Crocidolite asbestos and SV40 are cocarcinogens in human mesothelial cells and in causing mesothelioma in hamsters. Proc. Natl. Acad. Sci. U. S. A 103: 14128-14133.

Kurumatani,N. and S.Kumagai. 2008. Mapping the risk of mesothelioma due to neighborhood asbestos exposure. Am. J. Respir. Crit Care Med. 178: 624-629.

Landi,S., F.Gemignani, M.Neri, R.Barale, S.Bonassi, F.Bottari, P.A.Canessa, F.Canzian, M.Ceppi, R.Filiberti, G.P.Ivaldi, M.Mencoboni, P.Scaruffi, G.P.Tonini, L.Mutti, and R.Puntoni. 2007. Polymorphisms of glutathione-S-transferase M1 and manganese superoxide dismutase are associated with the risk of malignant pleural mesothelioma. Int. J. Cancer 120: 2739-2743.

Langerak,A.W., P.A.De Laat, Van Der Linden-Van Beurden CA, M.Delahaye, T.H.Van Der Kwast, H.C.Hoogsteden, R.Benner, and M.A.Versnel. 1996. Expression of platelet-derived growth factor (PDGF) and PDGF receptors in human malignant mesothelioma in vitro and in vivo. J. Pathol. 178: 151-160.

LaTulippe E., J. Satagopan, A. Smith, H. Scher, P. Scardino, V. Reuter, and W.L. Gerald. 2002. Comprehensive gene expression analysis of prostate cancer reveals distinct transcriptional programs associated with metastatic disease. Cancer Res 62:4499-506.

Lee,A.Y., D.J.Raz, B.He, and D.M.Jablons. 2007. Update on the molecular biology of malignant mesothelioma. Cancer 109: 1454-1461.

Leung,A.N., N.L.Muller, and R.R.Miller. 1990. CT in differential diagnosis of diffuse pleural disease. AJR Am. J. Roentgenol. 154: 487-492.

Lindbo,J.A., L.Silva-Rosales, W.M.Proebsting, and W.G.Dougherty. 1993. Induction of a Highly Specific Antiviral State in Transgenic Plants: Implications for Regulation of Gene Expression and Virus Resistance. Plant Cell 5: 1749-1759.

Lindholm,P.M., K.Salmenkivi, H.Vauhkonen, A.G.Nicholson, S.Anttila, V.L.Kinnula, and S.Knuutila. 2007. Gene copy number analysis in malignant pleural mesothelioma using oligonucleotide array CGH. Cytogenet. Genome Res. 119: 46-52.

Macura S.L., J.M.Hillegass, J.L.Steinbacher, M.B.MacPherson, A.Shukla, S.L.Beuschel, T.N.Perkins, K.J.Butnor, M.J.Lathrop, M.Sayan, K.Hekmatyar, D.J.Taatjes, R.A.Kauppinen, C.C.Landry, and B.T.Mossman. 2012. A multifunctional mesothelin antibody-tagged microparticle targets human mesotheliomas. J Histochem Cytochem 60(9):658-74.

Maroufi,A., B.E.Van, and L.M.De. 2010. Validation of reference genes for gene expression analysis in chicory (Cichorium intybus) using quantitative real-time PCR. BMC. Mol. Biol. 11: 15.

Matsuzaki,H., M.Maeda, S.Lee, Y.Nishimura, N.Kumagai-Takei, H.Hayashi, S.Yamamoto, T.Hatayama, Y.Kojima, R.Tabata, T.Kishimoto, J.Hiratsuka, and T.Otsuki. 2012. Asbestos-induced cellular and molecular alteration of immunocompetent cells and their relationship with chronic inflammation and carcinogenesis. J. Biomed. Biotechnol. 2012: 492608.

Mazzoni,E., A.Corallini, A.Cristaudo, A.Taronna, G.Tassi, M.Manfrini, M.Comar, M.Bovenzi, R.Guaschino, F.Vaniglia, C.Magnani, F.Casali, G.Rezza, G.Barbanti-Brodano, F.Martini, and M.G.Tognon. 2012. High prevalence of serum antibodies reacting with simian virus 40 capsid protein mimotopes in patients affected by malignant pleural mesothelioma. Proc. Natl. Acad. Sci. U. S. A 109: 18066-18071.

McDonald,J.C. and A.D.McDonald. 1996. The epidemiology of mesothelioma in historical context. Eur. Respir. J. 9: 1932-1942.

Melaiu,O., A.Cristaudo, E.Melissari, R.M.Di, A.Bonotti, R.Bruno, R.Foddis, F.Gemignani, S.Pellegrini, and S.Landi. 2012. A review of transcriptome studies combined with data mining reveals novel potential markers of malignant pleural mesothelioma. Mutat. Res. 750: 132-140.

Melotti A., A.Daga, D.Marubbi, A.Zunino, L.Mutti, and G.Corte. 2010. In vitro and in vivo characterization of highly purified human mesothelioma derived cells. BMC Cancer 10:54.

Metcalf,R.A., J.A.Welsh, W.P.Bennett, M.B.Seddon, T.A.Lehman, K.Pelin, K.Linnainmaa, L.Tammilehto, K.Mattson, B.I.Gerwin, et al. 1992. p53 and Kirsten-ras mutations in human mesothelioma cell lines. Cancer Res. 52: 2610-2615.

Metheny-Barlow,L.J., B.Flynn, H.E.van Gijssel, A.Marrogi, and B.I.Gerwin. 2001. Paradoxical effects of platelet-derived growth factor-A overexpression in malignant mesothelioma. Antiproliferative effects in vitro and tumorigenic stimulation in vivo. Am. J. Respir. Cell Mol. Biol. 24: 694-702.

Mohammad T., J.Garratt, E.Torlakovic, B.Gilks, and A.Churg. 2012. Utility of a CEA, CD15, calretinin, and CK5/6 panel for distinguishing between mesotheliomas and pulmonary adenocarcinomas in clinical practice. Am J Surg Pathol 36(10):1503-8.

Mohammed,K.A., X.Wang, E.P.Goldberg, V.B.Antony, and N.Nasreen. 2011. Silencing receptor EphA2 induces apoptosis and attenuates tumor growth in malignant mesothelioma. Am. J. Cancer Res. 1: 419-431.

Mohr S., G.Keith, F.Galateau-Salle, P.Icard, and B.H.Rihn. 2004. Cell protection, resistance and invasiveness of two malignant mesotheliomas as assessed by 10K-microarray. Biochim Biophys Acta 1688(1):43-60.

Moore,A.J., R.J.Parker, and J.Wiggins. 2008. Malignant mesothelioma. Orphanet. J. Rare. Dis. 3: 34.

Musti,M., D.Cavone, Y.Aalto, A.Scattone, G.Serio, and S.Knuutila. 2002. A cluster of familial malignant mesothelioma with del(9p) as the sole chromosomal anomaly. Cancer Genet. Cytogenet. 138: 73-76.

Mutsaers,S.E. 2004. The mesothelial cell. Int. J. Biochem. Cell Biol. 36: 9-16.

Mutsaers,S.E. 2002. Mesothelial cells: their structure, function and role in serosal repair. Respirology. 7: 171-191.

Narasimhan,S.R., L.Yang, B.I.Gerwin, and V.C.Broaddus. 1998. Resistance of pleural mesothelioma cell lines to apoptosis: relation to expression of Bcl-2 and Bax. Am. J. Physiol 275: L165-L171.

Ordonez,N.G. 2003. Application of mesothelin immunostaining in tumor diagnosis. Am. J. Surg. Pathol. 27: 1418-1428.

Ostroff,R.M., M.R.Mehan, A.Stewart, D.Ayers, E.N.Brody, S.A.Williams, S.Levin, B.Black, M.Harbut, M.Carbone, C.Goparaju, and H.I.Pass. 2012. Early detection of malignant pleural mesothelioma in asbestos-exposed individuals with a noninvasive proteomics-based surveillance tool. PLoS. One. 7: e46091.

Park,E.K., K.Takahashi, Y.Jiang, M.Movahed, and T.Kameda. 2012. Elimination of asbestos use and asbestos-related diseases: an unfinished story. Cancer Sci. 103: 1751-1755.

Pasello,G. and A.Favaretto. 2009. Molecular targets in malignant pleural mesothelioma treatment. Curr. Drug Targets. 10: 1235-1244.

Pass,H.I. and D.J.Mew. 1996. In vitro and in vivo studies of mesothelioma. J. Cell Biochem. Suppl 24: 142-151.

Pass,H.I., A.Wali, N.Tang, A.Ivanova, S.Ivanov, M.Harbut, M.Carbone, and J.Allard. 2008. Soluble mesothelin-related peptide level elevation in mesothelioma serum and pleural effusions. Ann. Thorac. Surg. 85: 265-272.

Peek,G.J., S.Morcos, and G.Cooper. 2000. The pleural cavity. BMJ 320: 1318-1321.

Peto,J., A.Decarli, V.C.La, F.Levi, and E.Negri. 1999. The European mesothelioma epidemic. Br. J. Cancer 79: 666-672.

Pietruska,J.R. and A.B.Kane. 2007. SV40 oncoproteins enhance asbestos-induced DNA double-strand breaks and abrogate senescence in murine mesothelial cells. Cancer Res. 67: 3637-3645.

Pomjanski,N., H.J.Grote, O.Sander, S.Heikaus, K.M.Müller, A.Böcking. 2008. Early cytological diagnosis of diffuse malignant mesothelioma of the peritoneum: a case report. Diagn Cytopathol. 36(2):120-3.

Powers,A. and M.Carbone. 2002. The role of environmental carcinogens, viruses and genetic predisposition in the pathogenesis of mesothelioma. Cancer Biol. Ther. 1: 348-353.

Price,B. and A.Ware. 2004. Mesothelioma trends in the United States: an update based on Surveillance, Epidemiology, and End Results Program data for 1973 through 2003. Am. J. Epidemiol. 159: 107-112.

Prins,J.B., K.A.Williamson, M.M.Kamp, E.J.Van Hezik, T.H.Van Der Kwast, A.Hagemeijer, and M.A.Versnel. 1998. The gene for the cyclin-dependent-kinase-4 inhibitor, CDKN2A, is preferentially deleted in malignant mesothelioma. Int. J. Cancer 75: 649-653.

Ramos-Nino,M.E., G.Vianale, T.Sabo-Attwood, L.Mutti, C.Porta, N.Heintz, and B.T.Mossman. 2005. Human mesothelioma cells exhibit tumor cell-specific differences in phosphatidylinositol 3-kinase/AKT activity that predict the efficacy of Onconase. Mol. Cancer Ther. 4: 835-842.

Rihn,B.H., S.Mohr, S.A.McDowell, S.Binet, J.Loubinoux, F.Galateau, G.Keith, and G.D.Leikauf. 2000. Differential gene expression in mesothelioma. FEBS Lett. 480: 95-100.

Rivera,Z., O.Strianese, P.Bertino, H.Yang, H.Pass, and M.Carbone. 2008. The relationship between simian virus 40 and mesothelioma. Curr. Opin. Pulm. Med. 14: 316-321.

Robinson,B.W., J.Creaney, R.Lake, A.Nowak, A.W.Musk, K.N.de, P.Winzell, K.E.Hellstrom, and I.Hellstrom. 2003. Mesothelin-family proteins and diagnosis of mesothelioma. Lancet 362: 1612-1616.

Robinson,B.W., A.W.Musk, and R.A.Lake. 2005. Malignant mesothelioma. Lancet 366: 397-408.

Robinson,C., B.van, I, A.Segal, M.Dunham, A.Sherwood, F.Koentgen, B.W.Robinson, and R.A.Lake. 2006. A novel SV40 TAg transgenic model of asbestos-induced mesothelioma: malignant transformation is dose dependent. Cancer Res. 66: 10786-10794.

Rodriguez Portal,J.A., B.E.Rodriguez, R.D.Rodriguez, M.Alfageme, I, M.A.Quero, R.C.Diego, J.A.Leon, M.Isidro, I, and R.P.Cebollero. 2009. Serum levels of soluble mesothelin-related peptides in malignant and nonmalignant asbestos-related pleural disease: relation with past asbestos exposure. Cancer Epidemiol. Biomarkers Prev. 18: 646-650.

Røe O.D., E.Anderssen, H.Sandeck, T.Christensen, E.Larsson, and S.Lundgren. 2010. Malignant pleural mesothelioma: genome-wide expression patterns reflecting general resistance mechanisms and a proposal of novel targets. Lung Cancer 67:57-68.

Røe O.D., E.Anderssen, E.Helge, C.H.Pettersen, K.S.Olsen, H.Sandeck, R.Haaverstad, S.Lundgren, and E.Larsson. 2009. Genome-wide profile of pleural mesothelioma versus parietal and visceral pleura: the emerging gene portrait of the mesothelioma phenotype. PLoS One. 4(8):e6554.

Rosen S.D., and H.Lemjabbar-Alaoui. 2010. Sulf-2: an extracellular modulator of cell signaling and a cancer target. Expert Opin Ther Targets 14:935-49.

Roushdy-Hammady,I., J.Siegel, S.Emri, J.R.Testa, and M.Carbone. 2001. Genetic-susceptibility factor and malignant mesothelioma in the Cappadocian region of Turkey. Lancet 357: 444-445.

Rundell,K. and R.Parakati. 2001. The role of the SV40 ST antigen in cell growth promotion and transformation. Semin. Cancer Biol. 11: 5-13.

Rusch,V.W., D.Giroux, C.Kennedy, E.Ruffini, A.K.Cangir, D.Rice, H.Pass, H.Asamura, D.Waller, J.Edwards, W.Weder, H.Hoffmann, J.P.van Meerbeeck; IASLC Staging Committee. 2012. Initial analysis of the international association for the study of lung cancer mesothelioma database. J Thorac Oncol. 7(11):1631-9.

Sandhu,H., W.Dehnen, M.Roller, J.Abel, and K.Unfried. 2000. mRNA expression patterns in different stages of asbestos-induced carcinogenesis in rats. Carcinogenesis 21: 1023-1029.

Sashital,D.G. and J.A.Doudna. 2010. Structural insights into RNA interference. Curr. Opin. Struct. Biol. 20: 90-97.

Sato M., L.Girard, I.Sekine, N.Sunaga, R.D.Ramirez, C.Kamibayashi, and J.D.Minna. 2003. Increased expression and no mutation of the Flap endonuclease (FEN1) gene in human lung cancer. Oncogene 22:7243-6.

Scherpereel,A., P.Astoul, P.Baas, T.Berghmans, H.Clayson, V.P.de, H.Dienemann, F.Galateau-Salle, C.Hennequin, G.Hillerdal, P.C.Le, L.Mutti, J.C.Pairon, R.Stahel, H.P.van, M.J.van, D.Waller, and W.Weder. 2010. Guidelines of the European Respiratory Society and the European Society of Thoracic Surgeons for the management of malignant pleural mesothelioma. Eur. Respir. J. 35: 479-495.

Scherpereel,A., B.Grigoriu, M.Conti, T.Gey, M.Gregoire, M.C.Copin, P.Devos, B.Chahine, H.Porte, and P.Lassalle. 2006. Soluble mesothelin-related peptides in the diagnosis of malignant pleural mesothelioma. Am. J. Respir. Crit Care Med. 173: 1155-1160.

Schipper,H., T.Papp, G.Johnen, H.Pemsel, R.Bastrop, K.M.Muller, T.Wiethege, M.Jaworska, M.Krismann, D.Schiffmann, and Q.Rahman. 2003. Mutational analysis of the nf2 tumour suppressor gene in three subtypes of primary human malignant mesotheliomas. Int. J. Oncol. 22: 1009-1017.

Schneider,J., H.Hoffmann, H.Dienemann, F.J.Herth, M.Meister, and T.Muley. 2008. Diagnostic and prognostic value of soluble mesothelin-related proteins in patients with malignant pleural mesothelioma in comparison with benign asbestosis and lung cancer. J. Thorac. Oncol. 3: 1317-1324.

Schwaller,B., I.Durussel, D.Jermann, B.Herrmann, and J.A.Cox. 1997. Comparison of the Ca2+-binding properties of human recombinant calretinin-22k and calretinin. J. Biol. Chem. 272: 29663-29671.

Seifert,R.A., C.E.Hart, P.E.Phillips, J.W.Forstrom, R.Ross, M.J.Murray, and D.F.Bowen-Pope. 1989. Two different subunits associate to create isoform-specific platelet-derived growth factor receptors. J. Biol. Chem. 264: 8771-8778.

Sekido,Y. 2008. Molecular biology of malignant mesothelioma. Environ. Health Prev. Med. 13: 65-70.

Selikoff,I.J., E.C.Hammond, and J.Churg. 1968. Asbestos exposure, smoking, and neoplasia. JAMA 204: 106-112.

Servais,E.L., C.Colovos, L.Rodriguez, A.J.Bograd, J.Nitadori, C.Sima, V.W.Rusch, M.Sadelain, and P.S.Adusumilli. 2012. Mesothelin overexpression promotes mesothelioma cell invasion and MMP-9 secretion in an orthotopic mouse model and in epithelioid pleural mesothelioma patients. Clin. Cancer Res. 18: 2478-2489.

Schmidmaier R., and P.Baumann. 2008. ANTI-ADHESION evolves to a promising therapeutic concept in oncology. Curr Med Chem 15:978-90.

Shield,P.W. and K.Koivurinne. 2008. The value of calretinin and cytokeratin 5/6 as markers for mesothelioma in cell block preparations of serous effusions. Cytopathology 19: 218-223.

Simeonova,P.P. and M.I.Luster. 1995. Iron and reactive oxygen species in the asbestos-induced tumor necrosis factor-alpha response from alveolar macrophages. Am. J. Respir. Cell Mol. Biol. 12: 676-683.

Singhal S., R.Wiewrodt, L.D.Malden, K.M.Amin, K.Matzie, J.Friedberg, J.C.Kucharczuk, L.A.Litzky, S.W.Johnson, L.R.Kaiser, and S.M.Albelda. 2003. Gene expression profiling of malignant mesothelioma. Clin Cancer Res 9(8):3080-97.

Spirtas,R., E.F.Heineman, L.Bernstein, G.W.Beebe, R.J.Keehn, A.Stark, B.L.Harlow, and J.Benichou. 1994. Malignant mesothelioma: attributable risk of asbestos exposure. Occup. Environ. Med. 51: 804-811.

Steinbach,D., M.Onda, A.Voigt, K.Dawczynski, S.Wittig, R.Hassan, B.Gruhn, and I.Pastan. 2007. Mesothelin, a possible target for immunotherapy, is expressed in primary AML cells. Eur. J. Haematol. 79: 281-286.

Stevens,J. and J.H.Rogers. 1997. Chick calretinin: purification, composition, and metal binding activity of native and recombinant forms. Protein Expr. Purif. 9: 171-181.

Stevenson L., W.L.Allen, I.Proutski, G.Stewart, L.Johnston, K.McCloskey, P.M.Wilson, D.B.Longley, and P.G.Johnston. 2011. Calbindin 2 (CALB2) regulates 5-fluorouracil sensitivity in colorectal cancer by modulating the intrinsic apoptotic pathway. PLoS One 6(5):e20276.

Storey,D. 2002. A direct approach to false discovery rates. J. R. Statist. Soc. B 64, Part 3, pp. 479–498.

Straif,K., L.brahim-Tallaa, R.Baan, Y.Grosse, B.Secretan, G.F.El, V.Bouvard, N.Guha, C.Freeman, L.Galichet, and V.Cogliano. 2009. A review of human carcinogens--part C: metals, arsenic, dusts, and fibres. Lancet Oncol. 10: 453-454.

Strickler,H.D., P.S.Rosenberg, S.S.Devesa, J.Hertel, J.F.Fraumeni, Jr., and J.J.Goedert. 1998. Contamination of poliovirus vaccines with simian virus 40 (1955-1963) and subsequent cancer rates. JAMA 279: 292-295.

Strizzi,L., A.Catalano, G.Vianale, S.Orecchia, A.Casalini, G.Tassi, R.Puntoni, L.Mutti, and A.Procopio. 2001. Vascular endothelial growth factor is an autocrine growth factor in human malignant mesothelioma. J. Pathol. 193: 468-475.

Stroobant,P. and M.D.Waterfield. 1984. Purification and properties of porcine platelet-derived growth factor. EMBO J. 3: 2963-2967.

Sudo,H., A.B.Tsuji, A.Sugyo, Y.Ogawa, M.Sagara, and T.Saga. 2012. ZDHHC8 knockdown enhances radiosensitivity and suppresses tumor growth in a mesothelioma mouse model. Cancer Sci. 103: 203-209.

Sugarbaker,D.J., W.G.Richards, G.J.Gordon, L.Dong, R.A.De, G.Maulik, J.N.Glickman, L.R.Chirieac, M.L.Hartman, B.E.Taillon, L.Du, P.Bouffard, S.F.Kingsmore, N.A.Miller, A.D.Farmer, R.V.Jensen, S.R.Gullans, and R.Bueno. 2008. Transcriptome sequencing of malignant pleural mesothelioma tumors. Proc. Natl. Acad. Sci. U. S. A 105: 3521-3526.

Sutton,A., H.Khoury, C.Prip-Buus, C.Cepanec, D.Pessayre, and F.Degoul. 2003. The Ala16Val genetic dimorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria. Pharmacogenetics 13: 145-157.

Taguchi,T., S.C.Jhanwar, J.M.Siegfried, S.M.Keller, and J.R.Testa. 1993. Recurrent deletions of specific chromosomal sites in 1p, 3p, 6q, and 9p in human malignant mesothelioma. Cancer Res. 53: 4349-4355.

Tamura,T., H.Minami, Y.Yamada, N.Yamamoto, T.Shimoyama, H.Murakami, A.Horiike, Y.Fujisaka, T.Shinkai, M.Tahara, K.Kawada, H.Ebi, Y.Sasaki, H.Jiang, and N.Saijo. 2006. A phase I dose-escalation study of ZD6474 in Japanese patients with solid, malignant tumors. J. Thorac. Oncol. 1: 1002-1009.

Tan,E., N.Warren, A.J.Darnton, and J.T.Hodgson. 2010. Projection of mesothelioma mortality in Britain using Bayesian methods. Br. J. Cancer 103: 430-436.

Tang Z., M.Feng, W.Gao, Y.Phung, W.Chen, A.Chaudhary, B.St Croix, M.Qian, D.S.Dimitrov, and M.Ho. 2013. A human single-domain antibody elicits potent anti-tumor activity by targeting an epitope in mesothelin close to the cancer cell surface. Mol Cancer Ther

Tang,Z., M.Qian, and M.Ho. 2013. The role of mesothelin in tumor progression and targeted therapy. Anticancer Agents Med. Chem. 13: 276-280.

Taniguchi,T., S.Karnan, T.Fukui, T.Yokoyama, H.Tagawa, K.Yokoi, Y.Ueda, T.Mitsudomi, Y.Horio, T.Hida, Y.Yatabe, M.Seto, and Y.Sekido. 2007. Genomic profiling of malignant pleural mesothelioma with array-based comparative genomic hybridization shows frequent non-random chromosomal alteration regions including JUN amplification on 1p32. Cancer Sci. 98: 438-446.

Testa,J.R., M.Cheung, J.Pei, J.E.Below, Y.Tan, E.Sementino, N.J.Cox, A.U.Dogan, H.I.Pass, S.Trusa, M.Hesdorffer, M.Nasu, A.Powers, Z.Rivera, S.Comertpay, M.Tanji, G.Gaudino, H.Yang, and M.Carbone. 2011. Germline BAP1 mutations predispose to malignant mesothelioma. Nat. Genet. 43: 1022-1025.

Testa,J.R. and A.Giordano. 2001. SV40 and cell cycle perturbations in malignant mesothelioma. Semin. Cancer Biol. 11: 31-38.

Teta,M.J., E.Lau, B.K.Sceurman, and M.E.Wagner. 2007. Therapeutic radiation for lymphoma: risk of malignant mesothelioma. Cancer 109: 1432-1438.

Thakur,S.A., R.F.Hamilton, Jr., and A.Holian. 2008. Role of scavenger receptor a family in lung inflammation from exposure to environmental particles. J. Immunotoxicol. 5: 151-157.

Tiedemann,K. 1976. On the yolk sac of the cat. Endoderm and mesothelium. Cell Tissue Res. 173: 109-127.

Tolnay,E., C.Kuhnen, T.Wiethege, J.E.Konig, B.Voss, and K.M.Muller. 1998. Hepatocyte growth factor/scatter factor and its receptor c-Met are overexpressed and associated with an increased microvessel density in malignant pleural mesothelioma. J. Cancer Res. Clin. Oncol. 124: 291-296.

Travis W.D., E.Brambilla, H.K.Muller-Hermelink, *et al*. 2004. WHO classification of tumours. Pathology and genetics of tumours of the lung, pleura, thymus, and heart. WHO classification of tumours. *IARCPress, International Agency for Research on Cancer: Lyon, France*, 128-136.

Tsao,A.S., D.He, B.Saigal, S.Liu, J.J.Lee, S.Bakkannagari, N.G.Ordonez, W.K.Hong, I.Wistuba, and F.M.Johnson. 2007. Inhibition of c-Src expression and activation in malignant pleural mesothelioma tissues leads to apoptosis, cell cycle arrest, and decreased migration and invasion. Mol. Cancer Ther. 6: 1962-1972.

Uehara,N., Y.Matsuoka, and A.Tsubura. 2008. Mesothelin promotes anchorage-independent growth and prevents anoikis via extracellular signal-regulated kinase signaling pathway in human breast cancer cells. Mol. Cancer Res. 6: 186-193.

Vaishnaw,A.K., J.Gollob, C.Gamba-Vitalo, R.Hutabarat, D.Sah, R.Meyers, F.T.de, and J.Maraganore. 2010. A status report on RNAi therapeutics. Silence. 1: 14.

Vandesompele,J., P.K.De, F.Pattyn, B.Poppe, R.N.Van, P.A.De, and F.Speleman. 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 3: RESEARCH0034.

Varin,E., C.Denoyelle, E.Brotin, M.Meryet-Figuiere, F.Giffard, E.Abeilard, D.Goux, P.Gauduchon, P.Icard, and L.Poulain. 2010. Downregulation of Bcl-xL and Mcl-1 is sufficient to induce cell death in mesothelioma cells highly refractory to conventional chemotherapy. Carcinogenesis 31: 984-993.

Ventii,K.H., N.S.Devi, K.L.Friedrich, T.A.Chernova, M.Tighiouart, E.G.Van Meir, and K.D.Wilkinson. 2008. BRCA1-associated protein-1 is a tumor suppressor that requires deubiquitinating activity and nuclear localization. Cancer Res. 68: 6953-6962.

Versnel,M.A., L.Claesson-Welsh, A.Hammacher, M.J.Bouts, T.H.Van Der Kwast, A.Eriksson, R.Willemsen, S.M.Weima, H.C.Hoogsteden, A.Hagemeijer, and . 1991. Human malignant mesothelioma cell lines express PDGF beta-receptors whereas cultured normal mesothelial cells express predominantly PDGF alpha-receptors. Oncogene 6: 2005-2011.

Virta RL. World Asbestos supply and consumption trends from 1900 through 2003. Available at: www.usgs.gov

WAGNER,J.C., C.A.SLEGGS, and P.MARCHAND. 1960. Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape Province. Br. J. Ind. Med. 17: 260-271.

Wang,K., V.Bodempudi, Z.Liu, E.Borrego-Diaz, F.Yamoutpoor, A.Meyer, R.A.Woo, W.Pan, A.Z.Dudek, M.S.Olyaee, T.Esfandyari, and F.Farassati. 2012. Inhibition of mesothelin as a novel strategy for targeting cancer cells. PLoS. One. 7: e33214.

Wang,Z.J., G.P.Reddy, M.B.Gotway, C.B.Higgins, D.M.Jablons, M.Ramaswamy, R.A.Hawkins, and W.R.Webb. 2004. Malignant pleural mesothelioma: evaluation with CT, MR imaging, and PET. Radiographics 24: 105-119.

Warren,S., C.E.Brown, R.N.Chute, and M.Federman. 1981. Mesothelioma relative to asbestos, radiation, and methylcholanthrene. Arch. Pathol. Lab Med. 105: 305-312.

Wheatley-Price,P., B.Yang, D.Patsios, D.Patel, C.Ma, W.Xu, N.Leighl, R.Feld, B.C.Cho, B.O'Sullivan, H.Roberts, M.S.Tsao, M.Tammemagi, M.Anraku, Z.Chen, P.M.de, and G.Liu. 2010. Soluble mesothelin-related Peptide and osteopontin as markers of response in malignant mesothelioma. J. Clin. Oncol. 28: 3316-3322.

Woo,H.G., E.S.Park, S.S.Thorgeirsson, and Y.J.Kim. 2011. Exploring genomic profiles of hepatocellular carcinoma. Mol. Carcinog. 50: 235-243.

Yamaguchi,N., K.Hattori, M.Oh-eda, T.Kojima, N.Imai, and N.Ochi. 1994. A novel cytokine exhibiting megakaryocyte potentiating activity from a human pancreatic tumor cell line HPC-Y5. J. Biol. Chem. 269: 805-808.

Yang,H., J.R.Testa, and M.Carbone. 2008. Mesothelioma epidemiology, carcinogenesis, and pathogenesis. Curr. Treat. Options. Oncol. 9: 147-157.

Yoshikawa,Y., A.Sato, T.Tsujimura, M.Emi, T.Morinaga, K.Fukuoka, S.Yamada, A.Murakami, N.Kondo, S.Matsumoto, Y.Okumura, F.Tanaka, S.Hasegawa, T.Nakano, and T.Hashimoto-Tamaoki. 2012. Frequent inactivation of the BAP1 gene in epithelioid-type malignant mesothelioma. Cancer Sci. 103: 868-874.

Yu,L., M.Feng, H.Kim, Y.Phung, D.E.Kleiner, G.J.Gores, M.Qian, X.W.Wang, and M.Ho. 2010. Mesothelin as a potential therapeutic target in human cholangiocarcinoma. J. Cancer 1: 141-149.

Zanella,C.L., J.Posada, T.R.Tritton, and B.T.Mossman. 1996. Asbestos causes stimulation of the extracellular signal-regulated kinase 1 mitogen-activated protein kinase cascade after phosphorylation of the epidermal growth factor receptor. Cancer Res. 56: 5334-5338.

Zhang,D., T.Kobayashi, T.Kojima, K.Kanenishi, Y.Hagiwara, M.Abe, H.Okura, Y.Hamano, G.Sun, M.Maeda, K.Jishage, T.Noda, and O.Hino. 2011. Deficiency of the Erc/mesothelin gene ameliorates renal carcinogenesis in Tsc2 knockout mice. Cancer Sci. 102: 720-727.

Zhao,Y.L., C.Q.Piao, L.J.Wu, M.Suzuki, and T.K.Hei. 2000. Differentially expressed genes in asbestos-induced tumorigenic human bronchial epithelial cells: implication for mechanism. Carcinogenesis 21: 2005-2010.

Zucali,P.A., G.L.Ceresoli, V.F.De, M.Simonelli, E.Lorenzi, L.Gianoncelli, and A.Santoro. 2011. Advances in the biology of malignant pleural mesothelioma. Cancer Treat. Rev. 37: 543-558.

Zucali,P.A. and G.Giaccone. 2006. Biology and management of malignant pleural mesothelioma. Eur. J. Cancer 42: 2706-2714.