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Genetic, Epigenetic and Molecular Changes in Melanoma: A New Paradigm for Biological Classification

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1. Introduction

The last two decades have registered a progressive decline of both the incidence and mortality rates for some human cancers, worldwide (Jemal et al., 2010). However, in the same time interval, the incidence of cutaneous melanoma (CM), has progressively increased (Gallagher et al., 2005; Jemal et al., 2010), up to an epidemic level in Western countries and Australia (Beddingfield et al., 2003; Cancer facts & Figures, 2009). To date, the average lifetime risk for developing melanoma ranges from 1/50 in the United States (men and women) (Meyle & Guldberg, 2009; Horner et al. 2011) up to 1/25 for Australian men (Hocker et al., 2008). CM is an extremely aggressive skin cancer, and constitutes one of the most lethal human malignancies, notwithstanding the progressive increase of early diagnosis and surgical excision registered from 1990s. This poor prognosis may be due, at least in part, to the possible occurrence of metastasis even in early phases of melanoma progression, as well as to the very low response to current systemic therapy. The 5 year-survival rate for patients with disseminated disease is about 10%, with death for disease ultimately occurring within 2 years from metastases (Balch et al., 2000; Chin et al., 2006; Zbytek et al., 2008; Ugurel et al., 2009; Cancer facts & Figures, 2009). This underscores the need to uncover the mechanisms underlying melanoma biology, with the aim to identify reliable early markers of response and to select patients eligible for new rational avenues for therapy (Siena et al., 2009). The formidable aggressive potential of CM is thought to represent the result of multiple intersecting molecular alterations of the control pathways governing cell proliferation, cell death, DNA-repair, and tumor-stromal interaction. These molecular alterations are thought to be involved also in the peculiar histomorphological features and different biological behavior of the four "classical" types of CM. Three of them (superficial spreading melanoma, SSM; malignant lentigo melanoma, LM; acral melanoma, AM) are characterized by the sequential progression from junctional (radial) and pagetoid (intraepithelial) growth phase, to invasive (vertical) growth. The radial growth phase (RGP) is characterized by lateral melanocyte growth at the dermo-epidermal interface (junctional area), whereas the vertical growth phase (VGP) shows the spreading of melanoma cells into the dermis and subcutis, this being correlated with the occurrence of metastasis. The fourth

type of CM, the "nodular" melanoma (NM), shows an *ab initio* invasive growth, and a more aggressive clinical course. Considerable insights have recently been made with respect to these topics. An impressive number of putative biomarkers of CM progression and drug response are being increasingly proposed, and new hypotheses concerning the interrelationship between histopathological features and clinical behavior are in progress. To date, however, we have to face with an "overload" of research data, which has already had a great impact on our understanding of melanoma progression, but is only beginning to unravel the real complexity of CM biology. Further work is required before we definitively know how we can use correctly these data to fight melanoma. We here review the more recent advances in molecular events involved in melanoma progression, drawing on a limited set of examples focused on the seminal findings of several research groups that have provided insight on melanoma biology. We discuss also the surprising interactions between some of these molecular pathways, both at genetic and epigenetic level, with particular attention to their role in defining potential markers for prognosis and therapeutic management of melanoma patients.

2. Genetics

Since the announcement of the completion of the Human Genome Project in 2003 (Noble, 2003), the first comprehensive analysis of cancer genomes has been completed: the entire genome of cutaneous melanoma and lung cancer has been sequenced. This formidable amount of information concerning the specific mutations of these cancer types will be of paramount importance in our understanding of the key molecular events that determine cancer evolution in every single patient. In tumors, most mutations are "passengers", meaning that they do not contribute to oncogenesis. Still, they provide information about the various steps leading to the oncogenic transformation of cells, as occurs, for example, for UV-exposure in skin cancer. This kinds of mutation, then, are very useful to study the pathogenesis of cancer. Only a small subset of somatic mutations is made of "driver mutations", which confer the oncogenic stigmata to cancer cells, being thus eligible for diagnosis and molecular therapy (Stratton et al, 2009). To date, more than 300 "driver" genes have been identified across all human cancers. Not surprisingly, they all converge over the few key cellular pathways which regulate cell life and differentiation (Vogelstein, 2010). The development and progression of melanoma is characterized by the acquisition of chromosomal deletions, amplification and gene mutations (Chin et al., 2006). The many gene expression profile studies performed up to now have evidenced that melanomas show significant genetic heterogeneity (Fecher et al., 2007; Ryu et al., 2007). Overall, large regions of the genome exhibiting DNA copy number changes and genes relevant for melanoma progression (Lin et al., 2008) as the Wnt5a, which misregulation results in increased motility in melanoma, have been identified (Lin et al., 2008). This also occurs for genes fundamental for basic melanocyte biology, such as Rab38, involved in melanosomal protein trafficking. We here refer to several genetic changes specifically linked to particular aspects of melanoma biology.

2.1 UVR

The dominant mutational signature emerging from sequencing the entire genome of cutaneous melanoma (Stratton et al, 2009) reflects DNA damage due to ultraviolet light exposure. Ultra-Violet Radiation (UVR) exerts direct or indirect damaging effects on nucleic

acids and proteins (Kyrgidis et al., 2010). UVA mutate DNA indirectly, via absorption by non-DNA endogenous sensitizers which generate Reactive Oxygen Species (ROS) responsible for DNA damage (Lund & Timmins, 2007). UVB directly cause two types of DNA lesions: cyclobutane pyrimidine dimers (CPDs), arising between adjacent thymine(T) or cytosine(C) residues, and -pyrimidine 4-pyrimidone photoproducts (6-4PP) . The ''UVB signature mutations" are characterized by CT→T and CC→TT transitions. Variants in MC1R, ASIP, TYR and TYRP1 have been identified as independent low-penetrance susceptibility genes for UV-induced melanomagenesis. These genes seem to contribute to the interrelation between pigmentation, cutaneous phototypes and exposure to UV light in affecting predisposition to melanoma (Thompson et al., 2009). Moreover, in melanomas of photoexposed skin, we have found defective expression of the mismatch-repair genes MHS2, which constitutes the phototype of mismatch-repair genes. It is actually thought that this may trigger microsatellite instability, thus contributing to the development of UV related skin tumors. In a previous study, we have shown also in oral melanomas that a deregulating expression of both hMSH2 and hMLH1 (mut L homologue 1) occurs. hMLH1 is frequently silenced in tumors with microsatellite instability. Both these genes can actually predispose to mutational events during tumor progression. We hypothesized that the altered expression of both HMSH2 and hMLH1 contributes to the mutagenic effect of UVR on melanocytes and, in turn, may be also involved in melanoma resistance to DNA damaging agents (Lo Muzio et al., 2000; Staibano et al., 2001).

2.2 BRAF

Alteration of the RAF/MEK/ERK pathway influences proliferation, invasion and survival of melanoma cells in vitro. For this reason it emerges then that B-Raf mutation has a pivotal role in determining melanoma biology, even if its exact function in melanoma progression remains still controversial. BRAF is a serine/threonine kinase that signals downstream of RTKs and RAS protein. Point mutations of B-Raf alter its auto-regulatory activation (Rother & Jones, 2009). The frequency of B-Raf mutations in melanoma and nevi is really impressive, being detectable in 30 to 80% of cases. Very interestingly, melanomas have a BRAF mutational spectrum different from other tumours, this probably reflecting the UVR environmental exposure (Thomas et al., 2006). This idea is further supported by the frequent occurrence of these mutations in SSM or NM of intermittently sun-exposed skin and in younger patients, whereas they are rare in AM (5-10%) and non-cutaneous melanomas (Platz et al., 2008). Over 30 distinct BRAF mutations, varying in biological activity, have been found and may be predictive of clinically relevant tumour differences (Thomas et al., 2006). The most common is constituted by a glutamic acid (glu) for valine (val) substitution at residue 599 (V600E) in the activation segment. At present, in spite of all these technical information, we are far away to the understanding of the real meaning of BRAF mutation in melanoma. It seems to correlate with distinct histopathologic features, such as intraepidermal "pagetoid" spreading of cohesive nests of malignant melanocytes, and "pushing" rather than infiltrative border of the tumor, as well as with the younger age and lymph node metastasis at diagnosis (Rother & Jones, 2009). However, these hypotheses show considerable inconsistencies, if we consider that benign nevi show even higher rates of V600E BRAF mutation and cell line or transgenic mouse models of melanoma have failed to unequivocally demonstrate the transforming ability of this mutation (Rother J et al, 2009). Recently, Dhomen N et al. have developed a mouse model of BRAFv600E-driven

melanoma, showing that V600E BRAF mutation alone stimulates proliferation of melanocytes but cannot induce full transformation, which presumably requires the acquisition of additional mutations (Dhomen N, et al., 2009). Nevertheless, the $BRAF^{V600E}$ has evolved into the most important target in melanoma. First-generation non-selective RAF inhibitor (Sorafenib) as a single therapy or in combination with carboplatin and paclitaxel (Hauschild et al., 2009), iinhibitors of the MAPK pathways with activity for either BRAF or MEK (as PLX4032, Flaherty et al., 2010), induction of specific cellular immunity against $BRAF^{V600E}$, $BRAF^{V600E}$ inhibitors based on the crystal structure of $BRAF^{V600E}$ complexes, are providing conflicting results in patients with metastatic melanoma. New potent small molecule inhibitors of mutant BRAF, combined to the immunotherapy agent ipilimumab have demonstrated promising clinical activity. However, despite initial high response rates, in most cases these results persist for a short time and melanomas recur after a few months, showing resistance to the previously effective B-RAFv600E inhibitors (Nazarian et al., 2010; Villanueva et al., 2010). One invoked mechanism which can influence the clinical response to antineoplastic therapy could be the pharmacogenomic variation in both the tumor and the patient's genome (Wang et al., 2011). Recently, high-throughput screening methods have been proposed to uncover the mechanisms of resistance in these tumors (Garraway, 2010). However, further studies on B-RAF mutant melanomas are needed before definitive results will be reached. A recent genome-wide RNA-interference screening targeting 28,000 genes, identified 17 genes able to block uncontrolled proliferation of melanocytes in the presence of BRAFV600E, among them a relevant role seems to be played by IGFBP7 (insulin-like growth factor binding protein 7), which is often epigenetically silenced in primary melanomas. The functional relevance of these genes in human melanoma remains unclear. Overall, it seems evident that several mechanisms act together during malignant transformation and progression of melanoma. Activating or inactivating mutations of members of the Akt signalling pathway (phosphatidylinositol 3-kinase CA, PI3KCA and Akt kinase) and mutations of tumor suppressor phosphatase and tensin homolog (PTEN) are frequently found in melanomas. Hopefully, targeting these molecular pathways will change the standard of care of metastatic melanoma in the future, but further studies focused on identifying predictors of chemotherapeutic response are needed.

2.3 PTEN/AKT

PTEN is a phosphatase that acts as a tumor suppressor gene, regulating the activation of the global regulators of cell proliferation serine/theronine kinases AKT1/2-3. Complete PTEN loss (usually due to genomic deletion) is found in 20-25% of melanomas, sometimes in combination with BRAF mutation, and leads to high-level AKT activation (Rother & Jones, 2009). Rare activating mutations of the AKT1 or AKT3 isoforms have been found in sun-exposed melanoma subtypes. Moreover, AKT overexpression may be associated with melanoma growth in situ.

2.4 Timing of melanoma progression

The central theme of melanoma research actually focuses on changes in gene expression occurring at the RGP/VGP shifting. We are now in a conceptual transition phase, in which the Clark model of neoplastic progression of melanocytes is being questioned. Basing upon the growing body of molecular data and considering the finding that many melanomas arise de novo on normal skin instead of from pre-exsisting melanocytic nevi, the biology of

melanocytic lineage could be examined from a point-of-view alternative to the postulated classical Clark model. This latter postulated that melanoma derives from (Takata et al., 2009) benign melanocytic nevus, which usually temporarily undergoes proliferation via oncogenic BRAF signalling, followed by growth arrest due to senescence induced by p16INK4/Rb. When a disruption of p16INK4a-retinoblastoma (Rb) pathway occurs, mostly by the inactivation of CDKN2A, the nevus become "dysplastic" and progressively more atypical, up to the RGP melanoma. At this stage, melanoma cells are dependent on growth factors secreted from keratinocytes, such as endothelin-1 (Murata et al., 2007), and are immortal, due to the activation of human telomerase reverse transcriptase (hTERT). In the vertical growth phase (VGP), mutations repressing apoptosis and favouring invasion (as PTEN loss, RAS activation, b-catenin activation) allow neoplastic melanocytes to survive in the absence of keratinocytes (Bennett 2003).
According to this theory, the interaction between melanoma cells and stromal fibroblasts further promotes tumor growth, migration, and angiogenesis (Li et al. 2003), but recently it has been proposed to revise this model of neoplastic progression of melanocytes, basing on the same evidences. Firstly, melanocytic nevi often consist of polyclonal population of either wild-type BRAF and BRAF mutated-nevus cells; moreover as before mentioned, many melanomas arise de novo (Takata et al., 2009). The p16INK4a-Rb inactivation (by deletion, mutation or promoter methylation of the CDKN2A gene, amplification of CCND1 or CDK4, or Rb mutation) (Bennett, 2008) has been demonstrated in early RGP melanomas (Takata et al., 2009), which in turn acquire a proliferative mutation, such as BRAFV600E, and clonally proliferate, maintaining a minimum telomere length through activation of hTERT. The full oncogenic transformation to VGP melanoma requires additional genetic or epigenetic changes, suppressing apoptosis, altering cell-cell adhesion, and leading to hyperproliferation and stromal invasion, for example via up-regulation of genes coding for cell surface and secreted proteins, as Neuropilin-2 (NRP2), which plays a critical role in mediating melanoma-endothelial interactions (Stine et al., 2011).

2.5 HIF1/ROS and melanoma-microenvironment

ROS are mutagenic molecules. Most of apoptosis regulators are known to be potentially mutated or functionally altered by ROS: BRAF (RAS, MEK, and ERK within the MAPK pathway), PTEN, Rb and AKT (Wittgen & van Kempen, 2007; Fruehauf & Trapp, 2008).
Melanoma cells generate large intracellular amounts of ROS (Sander et al., 2003) and excrete them into the extra cellular space. An upgraded ROS production has also been observed in dysplastic nevi (Pavel et al., 2004). ROS emerging as bio product during physiological melanin synthesis are neutralized within melanosomes by the anti-oxidant melanin. Melanosomes of malignant melanocytes produce excessive amounts of ROS (Gidanian et al., 2008; Josse et al., 2010) and in addition produce, instead of the regular eumelanin, pheomelanin, which is associated with more oxidative stress. In view of these unique melanoma properties, elevated production of ROS seems to be a melanoma- specific defect (Fruehauf and Trapp, 2008). Other factors may contribute to elevate ROS levels around the primary tumor: the skin is a hypoxic tissue, leading to ROS production (Wittgen & van Kempen, 2007) and exogenous attacks (e.g. UV-radiation) further increase oxidative stress. In addition, tumor-associated immune cells also excrete ROS (Nishikawa, 2008). Reactive oxygen species derived from immune cells have been proposed to exert a 'selective pressure' on MM cells to develop ROS-resistance (Wittgen & van Kempen, 2007). Following

this hypothesis, the entire 'ROS-saturated environment' in and around the primary tumor may exert a selective pressure on MM cells, causing the selection of those with the highest ROS resistance, whereas unfit cells die of ROS-induced apoptosis. After acquiring ROS resistance to block apoptosis, MM cells can also use high ROS levels to further stimulate their metastatic potential (Nishikawa and Hashida, 2006) through an impressive variety of pathways; from induction of DNA changes and activation of cell proliferation, to destruction of surrounding tissue, induction of adhesion molecules, activation of metastatic processes and escaping immune surveillance. In summary, ROS act as pro-metastatic agents through a wide range of pathways (Josse et al., 2010), which in melanoma cells accumulates early in tumor progression due to the alteration of melanin produced by characteristically abnormal melanosomes (Fruehauf & Trapp, 2008; Meyskens, 2001; Gidanian et al., 2008). HIF-1 activity is highly regulated by hydroxyl radicals and other reactive oxygen species (ROS) (Kietzmann & Gorlach, 2005; Wittgen & van Kempen, 2007, Kuphal et al., 2010) HIF-1 molecular complex is the major transcriptional regulator of the cellular and systemic response to a hypoxic environment, and is involved in cancerogenesis, regulating the expression of factors fundamental for angiogenesis (VEGF) and tumour invasion (glycolytic enzymes) (Forsythe et al., 1996; Wenger, 2002; Wiesener & Maxwell, 2003; Erler et al., 2006). HIF-1 contributes also to the induction of vasculogenic mimicry (Sun et al., 2007). In melanoma cells HIF-1 expression is also regulated both at the translational and transcriptional level by various other molecular ways, comprising the AKT/phosphatidylinositol 3-kinase (PI3K) pathway, (Jiang et al., 2001) enhanced by the hypoxic skin environment, and by V600E BRAF (Minet et al., 2000; Galabova-Kovacs et al., 2006) and the transcription factor NFkappaB (Bonello et al., 2007, Rius et al., 2008). This latter, in turn, is constitutively upregulated in melanoma cell lines (Kuphal et al., 2004) and, in addition, may be regulated by ROS and/or JNK, either induced by UVR or directly triggered by the activation of the RAS/RAF/MEK pathway (Tobar et al., 2008; Bonello et al., 2007; Li & Karin, 1999; Kuphal et al., 2010). HIF-1 activity is modulated also by mTOR. In particular, it has been shown that downregulation of HIF-1 by the mTOR inhibitor rapamycin prevents transformation under hypoxia, this suggesting that Rapamycin may be proposed as a therapeutic approach in melanoma treatment (Bedogni et al., 2005; Michaylira & Nakagawa, 2006). This is of particular interest, considering that Rapamicin has been shown to stimulate apoptosis in melanoma cell lines through the specific inhibition of FK506-binding protein 51 (FKBP51) isomerase activity (Romano et al., 2004).This protein belongs to a family of immunophilins physiologically expressed in lymphocytes, involved in the regulation of several fundamental biological processes and over-expressed in cancers and premalignant lesions. FKBP51 has been associated to the apoptosis resistance of malignant melanoma. In a recent study, we demonstrated that the expression of FKBP51 is markedly increased in vertical growth phase of melanomas and in metastatic lesions, providing evidence that FKBP51 is a marker of melanocyte malignancy (Romano et al., 2010a, 2010b). In addition we found that FKBP51 is a factor of resistance to genotoxic agents, including anthracyclins and ionizing radiations, through NF-kB activation.

2.6 cKIT

KIT encodes a receptor tyrosine kinase (RTK), which is recognized as a ligand for a stem cell factor (SCF) (Takata et al., 2009). Dysregulation of KIT plays a role in systemic mastocytosis, acute myelogenous leukaemia, gastrointestinal stromal cell tumors (GISTs) and germ cell tumors (Patnaik et al., 2007). A critical role for c-Kit for normal neural crest and normal

melanocyte development, (Rother & Jones, 2009) differentiation, proliferation, survival and migration (Wehrle-Haller, 2003) has been recognized, but its function in melanoma remains somewhat unclear. As a rule, c-Kit expression is lost during melanoma progression, but a subset of melanomas has been found to overexpress it and mutations activating c-Kit, mostly constituted by L576P (up to 50% of mutations), have recently been identified in some mucosal and AL melanoma subtypes (5-20% of cases), but not in cases arising from chronic sun-damaged skin (Rother & Jones, 2009; Monsel et al., 2010). KIT is expressed at maximum level at the invading edge of tumors, this suggesting a role for dynamic RTK activation in metastasis (Handolias et al., 2010). Monsel et al (Monsel et al., 2010) demonstrated that c-Kit mutated melanocytes require a specific epigenetic environment to be transformed in melanoma cells. c-Kit mutants cause in fact a strong activation of the phosphatidyl-inositol-3 kinase (PI3K) pathway, which, *per se*, is not sufficient to promote transformation of melanocytes. However, in the chronic hypoxic skin microenvironment, and/or when a constitutively active form of hypoxia-inducible factor 1alpha (HIF-1alpha) is coexpressed, c-Kit mutants activate also the Ras/Raf/Mek/Erk pathway, transforming the melanocytes, (Monsel et al., 2010). This scenario is extremely interesting, considering that KIT mutations are mutually exclusive with BRAF and NRAS, to identify a subset of melanomas arising from a distinct molecular mechanism of transformation, which may be specifically targeted by KIT inhibitors, such as imatinib or sorafenib (Monsel et al., 2010).

2.7 Plexin B1

Plexin B1 interacts with small G proteins to regulate cell proliferation, migration, and apoptosis, and is repressed by oncogenic B-RAF signalling; its loss of expression *in vivo* has been documented in breast cancer and renal cell carcinoma (Stevens et al., 2010). Plexin B1 functions as a tumor suppressor in melanoma cells, as it has been recently shown in a mouse model (Stevens et al., 2010; Argast et al., 2009). Introduction of plexin B1 into melanoma cell lines suppresses tumor formation in mice (Rody et al., 2009; Gomez Roman et al., 2008; Argast et al., 2009), this being thought to be due, at least in part, to suppression of c-Met signalling and c-Met-dependent migration. Recently, it has been evidenced that plexin B1 is lost in metastatic and deeply invasive melanoma in patient samples in vivo (Stevens et al., 2010), whereas it is generally hyperexpressed in benign nevi and thin melanomas. This finding suggests that the loss of plexin B1 contributes to late stages of melanoma progression, including invasion and metastasis. Recent evidences indicate that plexin B1 can also activates the PI3-kinase-Akt pathway, thus functioning as a tumor promoter and inhibitor of apoptosis in melanomas not driven by c-Met activation (Stevens et al., 2010). These results require further investigation. If confirmed, they could indicate that the final action of plexin B1 on melanoma progression may depend on the balance between c-Met receptor blockade and the interaction of plexin B1 receptor to downstream targets independent of c-Met signalling, such as Akt.

2.8 Tumor-stromal interactions

Mutant BRAF melanomas show high levels of constitutively activated Erk 1,2 (Houben et al., 2004). Consequences of Erk 1,2 pathway activation include induction of cell proliferation, expression of melanoma transcription factors, matrix metalloproteinases, specific integrin subunits, and resistance to apoptosis (Rubinstein et al., 2010). Melanoma chondroitin sulfate proteoglycan (MCSP) is a plasma membrane-associated proteoglycan which interacts with

distinct Erk-binding sites (Yang et al., 2009). As for Erk 1,2, its functions range from remodelling tumor microenvironments, facilitating the growth, invasion and motility of tumor cells to modify the organization of the cytoskeleton by modulating the activity of Rho family. Normal melanocytes express little or no MCSP, while the protein expression increases from benign and dysplastic nevi (Campoli et al., 2004) to melanoma. In RGP melanoma, MCSP expression enhances integrin function and constitutive activation of Erk 1,2. As already shown in acral lentiginous melanoma (such as in acute lymphoblastic leukaemia) MCSP expression is thought to be associated with a poor prognosis (Kageshita et al., 1991). These evidences indicate that MCSP, as a member of the Erk 1,2 pathway, may be considered as a promising therapeutic target in the treatment of melanoma.

3. Epigenetics

Epigenetics refers to changes in phenotype or gene expression without alterations of the DNA sequence. These changes occur at a much higher frequency compared to gene mutation, and may persist for the entire cell life and even for multiple generations (Kyrgidis et al., 2010). These "epimutations"allow the cell to modulate the transcriptional activity of a given gene, from high-level expression to complete silencing. Transcription results from the activity of the RNA polymerase machinery and depends on the ability of transcription activators and repressors to access chromatin at specific promoters (Santos-Rosa & Caldas, 2005). Chromatin is the dynamic scenario in which all the genomic functions take place; its structure is governed by the interplay of complex regulatory systems, which guarantee the maintenance of higher order chromosomal organization. Our understanding of how modulation of chromatin influences cell fate in normal as in neoplastic cells has been greatly improved during the last two decades. DNA replication, repair and transcription are achieved through the interplay of DNA modification and alteration in DNA packaging (Rountree et al., 2001). These mechanisms cooperate to establish the pattern of gene expression (Hashimshony et al., 2003) concerning every fundamental cell function, from cell growth and differentiation, to DNA repair and apoptosis. They operate through the induction of changes in chromatin architecture induced by histones (Dong & Bode, 2006) and "chromatin modifiers" (Kyrgidis et al., 2010). Histones are the main protein components of chromatin. Covalent post-translational modifications of the amino termini of the core histones in nucleosomes consist of methylation, acetylation, phosphorylation, ubiquitination and SUMOylation, and determine the "histone code" (Berger, 2002).

3.1 DNA methylation

DNA methylation occurs on lysine and arginine residues on histone tails. One preferred site of methylation is the C5 position of cytosine in the context of CpG dinucleotides. The latter may be found throughout the genome but are frequently clustered in short regions of 0.5–4kb, known as CpG islands (Bird, 2002), mostly located at the promoter regions of genes and unmethylated in normal cells. Hypermethylation of these regions causes the "silencing" of the target gene. DNA methylation is carried out by different DNA methyltransferases (DNMT) that use s-adenosyl-methionine as a methyl donor to replace a hydrogen atom with a methyl group (Board et al., 2008) and have distinct substrate specificities: DNMT1 (Pradhan et al., 1999), DNMT3a and 3b (Okano et al., 1999). All these DNMTs cooperate in establishing and maintaining DNA methylation patterns (Rhee et al., 2000; Kim et al., 2002).

In normal cells, methylation of CpG sites within promoter regions is used for controlling gene expression (Klose & Bird, 2006). In cancer cells, hypermethylation of the promoter regions of growth-regulatory genes leads to transcriptional silencing (Eden et al., 2003; Bonazzi et al., 2009), whereas hypo/demethylation results in de-repression of proto-oncogenes and genome wide hypomethylation leads to increased mutation rates and chromosome instability, which constitutes an early hallmark of tumour cells (Feinberg et al. 2002; Robertson, 2005; Martinez et al., 2009). The peculiar changes in DNA methylation patterns of human cancers are commonly constituted by reduced levels of DNA methylation and aberrantly hypermethylated CpG islands (Bird, 2002), which has been termed "the CpG island methylator phenotype" (CIMP) (Board et al., 2008). In cancer, epigenetic silencing through methylation occurs at even greater frequency than mutations or deletions and may be a more frequent cause of loss of function of tumor-suppressor genes than genetic defects (Jones & Baylin, 2002; Herman, 1999), suggesting that epigenetic changes have a major role in every step of tumor progression (Baylin, 2005). The genes that are known to be frequently hypermethylated and silenced in cancers frequently reside in chromosome regions that commonly show loss of heterozygosity, and their loss-of-function provides a selective growth advantage to neoplastic cells, and/or are implicated in tumor metastasis and angiogenesis (Baylin, 2005). Among these genes, we found most of them frequently mutated in cancers, as: ATM, ataxia telangiectasia mutated; APC, adenomatosis polyposis coli; BRCA1/2, breast cancer1/2; CDKN2A/B, cyclin-dependent kinase inhibitor 2A/B; GSTP1, glutathione S-transferase pi; MLH1, mutL homologue 1, colon cancer, non-polyposis type 2; MSH2, mutS homologue 2, colon cancer, non-polyposis type 1; NF12PTCHPTEN, phosphatase and tensin homologue; RB1, retinoblastoma 1; SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, member 3/subfamily B, member 1; TIMP3, tissue inhibitor of metalloproteinase 3; TP53/73, tumour protein p53/p73.

3.2 Histone acetylation

In an opposite manner respect to methylation, acetylation of histones reduces the positive charge, relaxing chromatin and making DNA in a transcriptionally active state, mediated mainly by the binding of transcriptional factors and histone acetylates. By a simplified point-of-view, we can assume that hyper acetylated histones are mostly associated with activated genomic regions, whereas deacetylation/hypoacetylation results in repression/silencing. In addition, histone acetylation allows also the binding of some regulatory transcriptional co-activators (Baylin, 2005), as the SWI/SNF adenosine triphosphatase (ATP)-dependent chromatin remodelling complex. Chromatin remodelling complex ("chromatin modifiers") is large protein complexes with enzymatic activity able to modify the structure and transcriptional activity of the chromatin. In an indirect manner, then, histone acetylation cooperates also to the regulation of gene expression mediated by the modification of higher-order chromatin folding (Verdone et al., 2006).

3.3 Phosphorylation

Other chromatin modifications, in concert with DNA methylation and acetylation, regulate gene transcription, in normal as in cancer cells, by affecting local chromatin structure. One of these modifications is constituted by phosphorylation of serines and threonines (Dong & Bode, 2006). Berger (Berger, 2002) showed that phosphorylation of histone (Ser10) is fundamental for neoplastic cell transformation.

3.4 Ubiquitination and SUMOylation

The other histone post-translational modifications include ubiquitination and SUMOylation. Ubiquitin is a highly conserved protein associated with most of fundamental cellular processes and its deregulated activity is thought to contribute to cancer onset or progression. SUMOylation directly affects nucleosomal structure, but the exact molecular mechanism is still not clear (Gill, 2004). Recently it has been shown that histone H4 is modified by SUMO, and SUMO-modification of histones has been suggested to contribute to transcriptional repression (Shiio & Eisenman, 2003), regulation of protein–protein interactions and activity of many factors involved in maintenance of the genome stability. Many factors and enzymes associated with DNA replication and repair including PCNA, and Topoisomerases I and II, are post-translationally modified by SUMO (Muller et al., 2004). The activity and/or localization of several tumor supressors and oncogenes are regulated by SUMO modification, and this has been hypothesized to provide new chances for new target therapy for cancer (Gill, 2004).

3.5 Interplay between different post-translational modifications

It has to be pointed out that single histone modifications may have distinct biological effects depending on their context. Methylation of H3 on K9, for example, is largely associated with silencing and repression. Methylation of lysine 9 on histone H3 is associated with epigenetically silenced chromatin, and loss of the H3K9me3 mark results in genomic instability (Cloos et al., 2006). Methylation of H3 on K4, by reverse, is most often associated with active or permissive chromatin regions. Moreover, demethylation of H3-K4 occurs at both inactive and active euchromatic genes, whereas tri-methylation is present exclusively at active genes. Similarly, the phosphorylation of H3 at S10 has been implicated not only in transcriptional activation, but also in mitotic chromosome condensation. This led to the suggestion that multiple readouts of a certain covalent mark could be obtained by various combinations of different modifications in the same chromatin region. Particular sets of modifications might occur concomitantly on the same histone tail. Mounting evidence suggests that different histone modifications can influence or 'communicate' with each other at several levels (Fischle et al., 2003). Moreover, as a rule, histone acetylation/deacetylation and methylation operate synergistically in cancer cells (Rountree et al., 2001), but competition for lysines may create crosstalk between SUMO and other modifiers. Acetylation may enhance SUMOylation of histone H4 (Gill, 2004); HATs and HDACs (that respectively add and remove acetyl groups) are post-translationally modified by SUMO, which regulates also their localization and/or activity (Girdwood et al., 2003). Cross-talk between the SUMOylation, ubiquitination, and acetylation occurs during signal-dependent regulation of several proteins (Hoege et al., 2002; Ross et al., 2002). From these data it emerges that the epigenetic framework which regulates gene expression is an extremely complex process, constituted by a multitude of intersecting molecular factors converging on chromatin.

3.6 miRNA

MicroRNAs (miRNAs) are recently discovered endogenous non-coding RNAs, about 22 nucleotide long (Guil & Esteller, 2009; Sigalotti et al., 2010). They play a role as mediators of epigenetic gene regulation, by interacting with mRNA, either by inhibiting mRNA translation or causing mRNA degradation (Sood et al., 2006). Their regulatory nature, as well as the large number of presumptive target genes, candidate them as regulators of several fundamental cellular processes, in normal as in cancer cells.

3.7 Melanoma epigenetics

3.7.1 Methylation

At present, it is not clear if UVR can initiate melanomagenesis through an epigenetic mechanism. Nevertheless, epigenetic changes, as mutations, increase after ultraviolet radiation (UVR) (Kyrgidis et al., 2010). This is of a great interest, considering that intermittent, intense exposure to UVR, especially during childhood, is major environmental risk factor in the etiology of melanoma (Maddodi & Setaluri, 2008). Deregulation of gene expression in melanoma progression requires epigenetic mechanisms which are currently uncompletely characterized, but have a potential great impact on the process of melanoma tumorigenesis and metastatic potential (Howell et al., 2009; Schinke et al., 2010). To date, more than 50 genes have been shown to be silenced through epigenetic changes during melanoma development, progression, and metastasis, mainly by promoter CpG island hypermethylation (Rothhammer & Bosserhoff, 2007; Howell et al., 2009), as it has been reported for MAGEA1 (Karpf et al.,2004). Most of these genes are not specific for melanoma, being involved in the control of cell cycle, cell signalling, immune recognition, angiogenesis, apoptosis, tumor cell invasion and metastasis in almost all cancer types (Rothhammer & Bosserhoff, 2007). Among these, there are CDKN2A, PTEN, APAF-1, TPM1 and TIMP3, several human leukocyte class I antigens and CASP8. Recently, have been found methylated in melanoma other genes linked to apoptosis (DAPKb, HSPB6, HSPB8, TMS1, TP53INP1, TRAILR1, XAF1), anchorage-independent growth (TPM1; cell cycle: CDKN1B, CDKN1C, CDKN2A, TSPY); chromatin remodeling (NPM2); differentiation (HOXB13, ENC1, GDF15); DNA repair (MGMT); invasion/metastasis (CCR7, CXCR4, SERPINB5); signaling (PGRbeta); transcription (RUNX3) (Sigalotti et al., 2010). In several cases, these aberrant hyper-methylations are recognizable in an high percentage of melanomas, as for Suppressors of cytokine signaling SOCS-1 (75%), SOCS-2 (75%), SOCS-3 (60%) (Liu et al., 2008), ER-α Estrogen receptor alpha (42%–86%) (Mori et al., 2006), p101(PIK3R5) Phosphoinositide-3-kinase, regulatory subunit 5 (88%), TNFRSF10C (DcR1) Tumor necrosis factor receptor superfamily, member 10c (55%) and TNFRSF10D (8 5%) (Liu et al., 2008), THBS4 Thrombospondin 4 (63%), RASSF1A RAS association domain family protein 1A (involved in apoptosis: its expression negatively correlates with lymph node metastasis, 55%), (Yi et al., 2010), HSP11, Heat shock protein H11 (60%), and RARβ2, Retinoic acid receptor, beta isoform 2 (70%, linked to the low retinoid sensitivity of melanoma) (Hoon et al., 2004). Up to 100% of melanoma cases show hyper-methylated CYP1B1 (Cytochrome P450, subfamily 1, polypeptide 1, linked to drug metabolism) and QPCT (Glutaminyl-peptide cyclotransferase) (Muthusamy et al., 2006). Recently, in cutaneous melanoma the loss of expression/methylation of gene promoter for Tumor suppressor in lung cancer 1 (TSLC1), encoding a member of the immunoglobulin superfamily, has been described (You et al., 2010). In some cancer types, this phenomenon leads to poor prognosis, and in melanoma it is significantly associated with advanced tumor stage and shorter disease-related survival, thus appearing as an important event in the pathogenesis of CM and a marker of poor prognosis (You et al., 2010). Similarly, recent findings indicated that a frequently aberrant methylated region in CM resides within the Zygote arrest 1 (ZAR1) gene, demonstrating a distinct methylation pattern between melanoma and nevus, thus hypothesizing that the aberrant methylation of ZAR1 may be a useful tumor biomarker to distinguish nevus from melanoma for early diagnosis (Shinojima et al., 2010). As well, there is an increasing body of evidence that, indicating the inactivation of the dimeric 14-3-3σ proteins member of the oncogene RAS/RAF/MEK/Erk pathway, involved in DNA damage

repair or arrest of cell cycle after severe damage, plays an important role in primary melanoma development. This has been reported for several malignant tumors (Schultz et al., 2009). Moreover, enhanced 14-3-3σ gene methylation in lymph node and cutaneous melanoma metastases compared with primary tumors was associated with significant 14-3-3σ downregulation. Treatment of melanoma cells with methylation and histone deacetylase inhibitors has led to the increase of 14-3-3σ expression, inhibition of cell proliferation, and induction of melanoma cell senescence. Epigenetic profiling showed, in addition, that multiple metastases after a single primary melanoma either share similar methylation patterns for many genes, or show differences in methylation between the lesions for several genes, as for PTEN, TFAP2C, and RARB (Harbst et al., 2010). Less is known on the presence and role of demethylation in CM, this reflecting the state-of-the art of the research on genome-wide demethylation in cancer, which has been overshadowed, to date, by studies of gene-specific hypermethylation events. It is generally thought that global demethylation early in tumorigenesis might predispose cells to genomic instability and further genetic changes, whereas gene-specific demethylation frequently constitutes a later event that favours the establishment of metastasis (Robertson, 2005). Recent study report that MAGE-A1, A2, A3, A4, which are involved in immune recognition and belong to the melanoma antigen family of cancer–testis genes, encoding tumour antigens of mostly unknown function, are frequently demethylated and re-expressed in cancer (Robertson, 2005) and hypomethylated in 44% of CM (MAGEA1) (Sigalotti et al., 2010; Tellez et al., 2009). This is very interesting, if we consider that the same family of genes, as before reported, may be found frequently hypermethylated in melanoma. This is also the case for the Testis-specific protein, Y-encoded (TSPY) (Oram et al., 2006; Howell et al., 2009), and maspin, a mammary serine protease inhibitor, acting as a TSG in breast cancer (Wada et al., 2004) that is repressed in normal melanocytes.

3.7.2 Phosphorylation

Phosphorylation of histone H3 at serine 28 and 10 is strongly induced by UV irradiation (Dong et al., 2006), and is mediated by the MAP kinase cascades (Zhong SP et al., 2000). UVA and UVB differentially induce the MAP kinase pathways at different levels (Bode & Dong, 2003): ERKs and p38 kinase mediate UVB-induced H3 (Ser10) phosphorylation in mouse epidermal skin cells and in vitro. It has been hypothesized that the p53 protein may directly regulate this phosphorylation by serving as a link or a bridge between these kinases and histone H3. Moreover, it has been found that also (He Z et al., 2005). Fyn, a member of the Src kinase family, and Aurora B kinase overexpression are involved in the UVB-induced phosphorylation of histone H3 (Ser10) (Ota et al., 2002). Recent data suggest that H3 histone phosphorylation plays a role in UV-mediated carcinogenesis.

3.7.3 Acetylation

Three members of the HATs family, the transcriptional coactivators, GNAT (general control non-depressible 5 (Gcn5)-related *N*-acetyltransferase), MYST (family members: MOZ, Ybf2-Sas3, Sas2, and Tip60) and p300/CBP-adenoviral E1A-associated protein, 300 kDa, (CREB-binding protein), have been recently found linked to melanoma progression. Members of the GNAT family (including GCN5, HAT1) and PCAF (p300/CREB-binding associated factor) acetylate histones H3 and H4 and non-histone transcriptional activators. P300 and CBP are global transcription coactivators that acetylate core histones and non-histone proteins such as p53, Rb, and E2F, involved in regulation of cellular proliferation, differentiation and apoptosis

(Shiama et al., 1997). Both CBP and p300 have been also shown to associate with microphthalmia-associated transcription factor (MITF), a melanocyte lineage survival oncogene (Garraway et al., 2005), which regulates melanoma proliferation, apoptosis and invasiveness (Dynek et al., 2008), and is mostly upregulated in metastatic melanomas (Lomas et al., 2008) being associated with decreased survival of patients (Ugurel et al., 2007).

3.7.4 Ubiquitination/SUMOylation

Accumulating evidence confirms the involvement of ubiquitin and ubiquitin–related pathways in melanomagenesis. Transcription factors such as MiTF, beta-catenin and IkB, which regulate the proliferation and differentiation of melanocytes, are subjected to this process (Wu et al., 2000). In normal melanocytes, MITF may be in turn subjected to ubiquitination or SUMOylation. In melanoma, ubiquitination leads to the corresponding elevated activity of MITF (Nakayama, 2010). In addition, melanoma cells show an alterated ubiquitination-dependent degradation of IKB, with consequent high rate of nuclear translocation of free NF-kB and activation of its target genes (Nakayama, 2010). Notably, also B-catenin is a target of the ubiquitin–proteasome pathway in melanoma. Several beta-catenin mutants lack sites of ubiquitination and, consequently, become stabilized because of the inhibition of proteasome-mediated proteolysis; thus promoting proliferation and survival of melanoma cells (Aberle et al., 2007; Rubinfeld et al., 1997; Widlund et al., 2002).

3.7.5 Chromatin texture

The neoplastic growth-induced modification of composition and distribution of histone and non-histone nuclear proteins provokes alterations of the distribution of heterochromatin in the nucleus. This reflects on the characteristics of the nuclear architecture, as seen on routinely stained histopathological sections, and has suggested as prognostic markers in cancer (Montironi et al., 2007). The determination of fractality characterizes the complexity of a structure not revealed by classical morphometry. The fractal nature of nuclear chromatin and of the surrounding nucleoplasmic space (Lieberman-Aiden et al., 2009; Bancaud A et al., 2009) are of prognostic importance in neoplasias (Goutzanis et al., 2008; Mashiah et al., 2008). Unstable, aggressive tumors with a high number of genetic or epigenetic changes, are characterized by a complex chromatin rearrangement, with an increased number of darker and lighter areas. The increased nuclear fractal dimension found in the most aggressive melanomas is the mathematical equivalent of this complex chromatin architecture, constituting a new and promising variable in prognostic models (Bedin et al., 2010), able to provide prognostic information independent from the invasion level of melanomas.

3.7.6 Chromatin assembly

Nuclear structure and function interact reciprocally at all the three hierarchical nuclear levels (coordination of nuclear processes, higher-order chromatin fiber organization, and spatial arrangement of genome) (Misteli, 2007). The chromatin template undergoes structural reorganizations during DNA replication and cell cycle progression (Fischle et al., 2003). Nucleosomes, made of 146 base pairs of DNA wrapped around a histone octamer core comprising two copies each of histones H2A, H2B, H3 and H4, represent the basic functional units of chromatin. Each histone contains flexible N-terminal tails protruding from the nucleosomes, which are extensively targeted by post-translational modifications, including acetylation and methylation (Sigalotti et al., 2010). When in open configuration,

they allow access of transcription factors. By converse, when they are in a hyper-compacted state, as in heterochromatin, inhibit transcription (Jones & Baylin, 2002; Jenuwein & Allis, 2001; Noma et al., 2001). Nucleosome structure and histone acetylation/methylation equally affect chromatin structure, regulating gene transcription (Baylin, 2005). Among molecules responsible of the chromatin assembly, a pivotal role is exerted by the Chromatin Assembly Factor-1 (CAF-1), a protein complex, formed of three subunits with different molecular weight: p48, p60 and p150. CAF-1 deliveres histones H3 and H4 to DNA during DNA replication and DNA repair, and CAF-1/p60 has been found overexpressed in a series of human malignancies, including breast, prostate, oral squamous and salivary glands cancers, in close association with their biological aggressiveness. We have evaluated by immunohistochemistry the expression of CAF-1/p60 in a selected series of cutaneous melanomas paraffinized tissue (Mascolo et al., 2010), comparing results with the clinical and pathological features of each tumor and with patient's outcome. We found a significant association between hyperexpression of CAF-1/p60 and the occurrence of node and/or distant metastases in SM patients. These findings indicate that CAF-1 may have a role as novel, sensible proliferation and prognostic marker for CM. CAF-1 overexpression can be easily evaluated by immunohistochemistry on formalin-fixed, paraffin-embedded tissue, and may constitute a potential useful adjunctive tool for pathologists for predicting the individual prognosis of melanoma patients.

3.7.7 Remodeling proteins

Recent studies have shown that SWI/SNF ATP dependent chromatin remodeling enzymes are involved in the molecular alterations occurring in melanoma (Saladi et al., 2010) and regulate the expression of genes important for tumor metastasis. SWI/SNF enzymes promote neural crest migration and differentiation and interact with Microphthalmia – Associated Transcription Factor (MITF), a known lineage survival oncogene in melanoma (de la Serna et al., 2006; Matsumoto et al., 2006; Saladi et al., 2010). SWI/SNF are multisubunit complexes that contain either BRG1 or BRM as the catalytic subunit (Li B et al., 2007) regulating important aspects of melanoma phenotype. While BRM expression is variable in melanoma (cell lines), BRG1 expression is increased at the protein levels in primary melanoma tumors compared to dysplastic nevi, and in most melanoma cell lines (Keenen et al., 2010; Vachtenheim et al., 2010). A BRG1 expression increases during melanoma progression and is thought to play an important role in melanoma metastasis, as it promotes melanoma invasive ability in vitro. This is supported by the ability of BRG1 to modulate the expression of a subset of cell surface receptors, adhesion proteins, and extracellular matrix remodeling enzymes in normal as in cancer cells, interacting with a transcriptional regulator of MMP2, the SP1 transcription factor, being also recruited to the matrix metalloproteinase (MMP2) promoter. However, up to date there was found no significant difference in BRG1 levels between primary and metastatic melanoma samples, but there has been evidenced a tendency for negative to weak BRG1 expression in the cases with a better patient survival (Lin et al., 2010).

3.7.8 miRNA

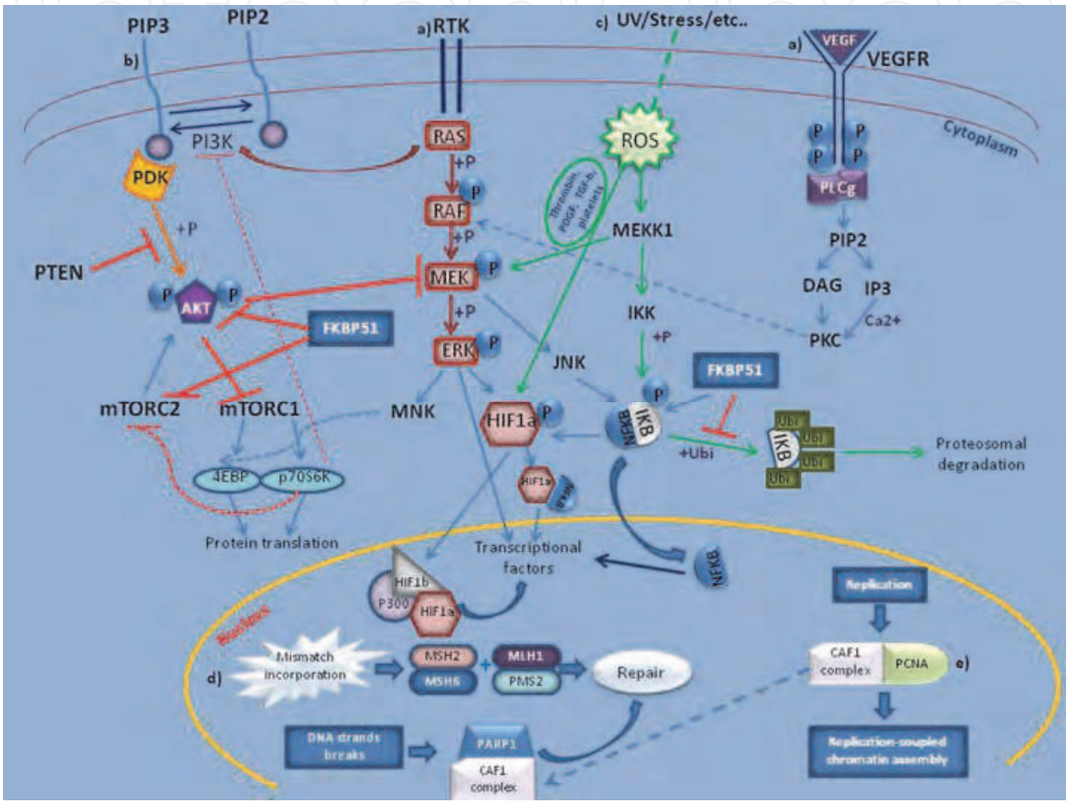
As before outlined, microRNAs (miRNAs) may act as either oncogenes or tumor suppressor genes. Recently, it has been shown that miRNAs act as tumor suppressors in uveal melanoma cells through downregulation of MITF and CDK6, and, particularly, that miR-137

is frequently silenced in this tumor (Chen et al., 2011). Up to now, limited data are available on miRNA deregulation in CM, almost all relative to small series of cases (Mueller et al., 2009; Sigalotti et al., 2010). However, a recent specific miRNA profiling study reports extensive modifications of miRNA patterns in CM as compared to normal melanocytes, and identifyies modifications of miRNA expression potentially associated to the different phases of CM progression (Mueller et al., 2009). Another study reports that miR-17-5p, miR-18a, miR-20a and miR-92a are over-expressed, while miR-146a, miR-146b, and miR155 are down-regulated in the majority of the examined CM cell lines as compared to normal melanocytes (Levati et al., 2009). Of particular interest, the master regulator of melanocytes biology, transcription factor MITF, has been found to be regulated by at least 2 different miRNAs, miR-137 and miR-182, which showed opposite alterations (Bemis et al., 2008). Other miRNAs have been found overexpressed in CM cell lines and tissues. Among them, miR-182 appeared to be involved in CM progression, being increasingly over-expressed from primary to metastatic disease. Its action has been related to the repression of MITF and FOXO3 (Segura. et al., 2009). The overexpression of miR-29c has been recently reported to be inversely correlated to DNMT3A and DNMT3B protein expression (Nguyen et al., 2011) and predictive of overall survival in AJCC stage III melanoma patients by multivariate analysis. On the contrary, members of the let-7 family of miRNAs are significantly downregulated in primary melanomas compared with benign nevi (Schultz et al., 2008). As in normal cells let-7 miRNAs exhert a regulatory activity on the expression of cyclins D1, D3, and A, as well as cyclin-dependent kinase (Cdk) 4, all of which have been described to play a role in melanoma development, (Müller & Bosserhoff, 2008). It appears evident that the loss of let-7a expression may contribute in the development and progression of melanoma.

3.7.9 Epigenetic drugs

DNA methylation inhibitors (as azacitidine and decitabine), along with inhibitors of histone deacetylation, have improved the therapeutic options of some cancer types, and particularly of several hematologic malignancies, where gene hypermethylation typically occurs (Baylin, 2005). The potential reversibility of epigenetic changes via pharmacologic manipulation, confers to this area of research particular relevance for the alternative therapeutic treatment of patients with advanced melanoma. New agents affecting epigenetic alterations in melanoma are thus being increasingly produced. The treatment of CM with these "epigenetic drugs" generally produce multiple effects, due to the reactivation and/or suppression of many of intersecting pathways that became altered during melanoma progression (Sigalotti et al., 2007). There is a tendency to combine epigenetic intervention with conventional and/or innovative therapeutic approaches that would take specific advantages from the epigenetically-restored pathways. As an example, strategies to restore the loss of expression of spindle checkpoint proteins, such as RASSF1A, by use of demethylating agents may be of utility in reversing genetic instability associated with melanoma progression (Rother & Jones, 2009). Indeed, in advanced stage melanoma, RASSF1A appears to correlate at some degree with response to chemotherapy. As a result, profiling of the activation state or degree of mitotic spindle dysfunction using these markers, shows promise in identifying those patients who would benefit from spindle toxins. The drugs typically used as multi-agent chemotherapy regimens to treat melanoma include carboplatin and cisplatin, alkylating agents, and mitotic spindle poisons such as

vinblastine and paclitaxel. Since therapeutic activity of alkylating agents and DNA-damaging agents requires tumor cell division, melanomas with high proliferative rate or those with genetic alterations in checkpoint function may be more likely to respond (Rother & Jones, 2009). Similarly, a particular clinical benefit might be expected from the synergistic effect of the pharmacologic inhibition of DNA methyltransferases and/or of histone deacetylases with chemo-, radio-, and immuno-therapeutic approaches in melanoma patients (Sigalotti, 2010). The introduction of new epigenetic drugs in this context is



a) RTK-RAS-RAF-MEK-ERK pathway. When activated, RAS triggers a phosphorylation cascade of RAF, MEK and ERK. MEK, when phosphorylated, activates Jun N-terminal kinase (JNK) cascade; ERK translocates to the nucleus, where it activates transcription factors for cell proliferation. VEGFR is a RTK and, therefore, when activated, leads to activation of this pathway. b) PI3K-AKT pathway. Activation of RTKs leads to activation of PI3K and, subsequently, of PDK1 that phosphorylates and activates AKT. AKT regulates several proteins affecting cell growth and survival. c) ROS signaling pathway. ROS production can induce HIF-1a (hypoxia-inducible transcription factors 1) under normoxia in response to some factors. Intracellular ROS activates also the NFkB (nuclear factor-kappa route B). d) DNA-repair pathways. PARP1 (Poly [ADP-ribose] polymerase 1) has a role in repair of single-stranded DNA (ssDNA) breaks, modifying nuclear proteins by polyADP-ribosylation. Component of the post-replicative DNA mismatch repair system (hMSH2 and hMLH) binds to DNA mismatches thereby initiating DNA repair. e) The Chromatin Assembly Factor-1 (CAF-1) promotes also the first step of nucleosome assembly during DNA replication, driving the incorporation of different histones into DNA to the sites where chromatin has to be newly formed or remodeled. CAF-1 promotes chromosome assembly during resetting of the chromatin structure after DNA repair (d).

Fig. 1. Crosstalks between signaling pathways involved in melanomagenesis and progression

GENETIC CHANGES	
Mutations	ASIP, β-catenin, BRAF, CCND1, CDKN2A, CDK4, Rb, cKIT, HIF1/ROS, IGFBP7, MC1R, NEUROPILIN2, NFkB, NRAS, PI3KCA, p16INK4, PLEXIN B1, PTEN/AKT, Rab38, TYR, TYRP1, WNT5A
EPIGENETIC ALTERATIONS	
Hypermethylation	ATM, APAF-1, APC, BRCA1, BRCA2, CASP8, CCR7, CXCR4, CDKN1B, CDKN1C, CDKN2A, CDKN2B, CYP1B1, DAPKb, ENC1, ER-α, GDF15, GSTP, H3, HLA class I, HOXB13, HSPB6, HSPB8, HSP11, MAGE-A1, MGMT, MLH1, MSH2, NF12PTCHPTEN, NPM2, p101(PIK3R5), PGRbeta, PTEN, QPCT, RARβ2, RASSF1A, RB1, RUNX3, SERPINB5, SOCS1, SOCS2, SOCS3, Stratifin (14-3-3sigma), SWI/SNF, TFAP2C, THBS4, TIMP3, TMS1, TNFRSF10C (DcR1), TNFRSF10D, TP53INP1, TP53/73, TPM1, TRAILR1, TSCL1, TSPY, XAF1, ZAR1
Hypomethylation	MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, Maspin, TSPY,
Phosphorylation	H3 (ser 28), H3 (ser 10)
Acethylation	E2F, H3, H4, MITF, p53, Rb, SWI/SNF (ATP)-dependent chromatin remodelling complex,
Ubiquitination/SUMOylation	H4, PCNA, Topoisomerase 1, Topoisomerase 2, MiTF, IkB, β-catenin
Chromatin assembly	Chromatin Assembly Factor-1 (CAF-1)
Remodelling proteins	SWI/SNF chromatin remodelling enzymes
miRNA overexpressed	miR-29c, miR-17-5p, miR-18a, miR-20a, miR-92a, miR-182
miRNA downregulated	miR137, miR-146a, miR-146b, miR155, miRNA let-7a
Tumor-stromal interactions	MCSP/NG2

Table 1. Summary of major genetic and epigenetic alterations in melanoma.

expected to reduce systemic toxicities of traditional therapies. Histone deacetylase (HDAC) inhibitors represent a promising therapeutic option for melanoma treatment (Facchetti F et al., 2004) and are particularly interesting with this regard. It has been shown, in fact, that HDAC inhibitors enhance the response of human tumor cells to ionizing radiation (Munshi et al., 2006) and synergize with radiation, reducing the clonogenic survival of CM cells. This beneficial effect seems to be related to their ability to sensitize CM cells to radiation-induced apoptosis, impairing also the ability of CM cells to repair DNA damages through the down-regulation of the repair proteins Ku70, Ku80, Ku86 and Rad50) (Munshi et al., 2006). These exciting results support the idea that combined epigenetic chemo/radiotherapies might overcome the resistance of CM to traditional therapies. In addition, HDAC inhibitors have been shown to sensitize melanoma cells to retinoid treatment, leading to the increase of tsg p16INK4A, either mutated or with homozygous deletion in most of late-stage melanoma

cells, thus correlating with their increased senescence. A further interesting example of possible integration between "old" and "new, epigenetic" therapies, is offered by the major polyphenolic constituent of green tea, the epigallocatechin-3-gallate (EGCG), which has anti-proliferative, pro-apoptotic and chemopreventive effects against cancer cells, including melanoma (Nihal et al., 2010), mediated through its demethylating activity. EGCG sensitizes melanoma cells to interferon in mouse models of human melanoma, by down-regulating NF-κB promoter activity, induced by reactive oxygen species (Fried & Arbiser, 2008). EGCG synergizes with the HDAC inhibitory action of vorinostat to help de-repress silenced tumor-suppressor genes regulating key functions, such as proliferation and survival. Lastly, it has to be outlined the potential beneficial effect of therapeutically restoring miRNA activity in CM.

4. Conclusions

Cross-talk between different signaling cascades has emerged as a paradigm of cell biology, in that they direct the local and global cell functions (Fischle et al., 2003). The epigenetic code is made of post-translational marks which correlate with specific transcriptional states and may influence one another (Berger, 2002), their combinations ultimately directing downstream functions. We are beginning to unravel the complexities of gene expression, which is conditioned by a myriad of diverse stimuli, acting in a multidimensional fashion (Dong & Bode, 2006), with histone modification and ATP-dependent chromatin remodeling being functionally connected for gene regulation. DNA methylation is controlled at several different levels in normal and tumor cells, directly by DNA methyltransferases (DNMTs), and indirectly by nucleosome spacing and histone deacetylases (HDACs) (Baylin, 2005; Bird A, 2002; Bird & Wolffe, 1999). Synergism/antagonism of adjacent modifications in the same histone tail is the rule ('cis' effects), but modifications on different histones affect each other ('trans' effects) (Fischle et al., 2003). Overall, it is now clear that each specific genetic or epigenetic alteration may impact the biology of CM cells by concurrently affecting multiple proteins/pathways. Further study will determine which panels of genes are most effective as biomarkers for prediction of response to therapy and prognosis and may be translated into clinically applicable tests. The different methylation or acetylation patterns may indicate important differences in tumorigenesis and may help identify subsets of patients with different clinical behaviour. The reported correlation of alterations in tumour DNA with circulating tumour DNA allows the basis for the development of biomarker blood tests for early detection of melanoma metastasis and prediction of response to therapy. Being a relatively rare event in normal melanocytes, specific hypermethylations of CpG islands are ideal candidate as biomarkers for early melanoma metastasis detection in body fluids (Howell et al., 2009). In line with this hypothesis, it has been recently reported that estrogen receptor A (ER-A) methylation can predicts melanoma progression, and serum methylated ER-A constitutes an unfavorable prognostic factor and negative predictor of progression-free survival in patients treated with biochemotherapy (Mori et al., 2006; Satzger I, 2009). Many studies are investigating the methylation status of several genes in sera of CM patients (Satzger I, 2009). We have now summarized only a selection of the hot topics concerning the genetic and epigenetic alterations that contribute to the development and progression of melanoma. Many questions remain unanswered. Cancer research is moving away from an histology-based view of cancer toward a genomic concept of the neoplastic disease (Garraway, 2010), and therapy is shifting toward pharmacogenomics, engineered to hit the specific genetic and epigenetic profile (the "genomic grade") of the single tumor in

each single patient, (Kim et al., 2010; Piccart-Gebhart, 2010). These goals are necessary for ultimately improve melanoma patient outcomes and increase drug efficacy against this lethal cancer. Further work is required to improve our understanding of the precise role of interlinked modifications that dictate specific genomic states, such as gene activation, repression, DNA repair in melanoma progression. Bioinformatics-based tools are being currently used to generate complex fingerprints based on a combination of genetic and epigenetic markers. Recent mathematical modeling of the dynamics of transcriptional control of gene expression will be relevant to a full understanding of MITF, SOX10, and PAX3 functions (Ben-Tabou de-Leon & Davidson, 2009). An unconditioned approach to the importance of the yet known key molecules involved in melanocyte development and function, under the light of the growing body of newly discovered interrelationships between new and old members of the most relevant pathways involved in melanoma progression, will be fundamental to improve the chances of the pigment research community to fight melanoma. The shift occurring towards personalized therapy in cancer is also beginning to be seen in melanoma, and new generation epigenetic drugs are be expected to reduce systemic toxicities with more specific effects (Sigalotti et al., 2010), even if additional pre-clinical studies are required to define even more precisely their consequences on normal cells and to predict their safety when used on patients. Convincing evidence that we are on the right way to truly understand melanoma biology already exists. A comprehensive point-of-view, considering simultaneously the phenotype, genotype and epigenotype of melanoma cells with respect to the differences in clinical behavior (Shackleton & Quintana, 2010) will help us to guide treatment and target those patients with specific, personalized therapies.

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6. References

Aberle, H., Bauer, A., Stappert, J., Kispert, A., & Kemler, R. (1997). beta-catenin is a target for the ubiquitin-proteasome pathway. *EMBO J*, Vol. 16, No. 13, (Jul 1997), pp. 3797-3804, ISSN 0261-4189

Argast, G.M., Croy, C.H., Couts, K.L., Zhang, Z., Litman, E., Chan, D.C., & Ahn, N.G. (2009). Plexin B1 is repressed by oncogenic B-Raf signaling and functions as a tumor suppressor in melanoma cells. *Oncogene*, Vol. 28, No. 30, (Jul 2009), pp. 2697-2709, ISSN 0950-9232

Balch, C.M., Buzaid, A.C., Atkins, M.B., Cascinelli, N., Coit, D.G., Fleming, I.D., Houghton, A. Jr, Kirkwood, J.M., Mihm, M.F., Morton, D.L., Reintgen, D., Ross, M.I., Sober, A., Soong, S.J., Thompson, J.A., Thompson, J.F., Gershenwald, J.E., & McMasters, K.M. (2000). A new American Joint Committee on Cancer staging system for cutaneous melanoma. *Cancer*, Vol. 88, No. 6, (Mar 2000), pp. 1484-1491, ISSN 1097-0142

Bancaud, A., Huet, S., Daigle, N., Mozziconacci, J., Beaudouin, J., & Ellenberg, J. (2009). Molecular crowding affects diffusion and binding of nuclear proteins in

heterochromatin and reveals the fractal organization of chromatin. *EMBO J*, Vol. 28, No. 24, (Dec 2009), pp. 3785-3798, ISSN 0261-4189

Bauer, J., & Bastian B.C. (2006). Distinguishing melanocytic nevi from melanoma by DNA copy number changes: comparative genomic hybridization as a research and diagnostic tool. *Dermatol Ther*, Vol. 19, No. 1, (Jan-Feb 2006), pp. 40-49, ISSN 1529-8019

Baylin, S.B. (2005). DNA methylation and gene silencing in cancer. *Nat Clin Pract Oncol*, Vol. 2, Suppl 1, (Dec 2005), pp. S4-11, ISSN 1743-4262

Beddingfield, F.C. 3rd. (2003). The melanoma epidemic: res ipsa loquitur. *Oncologist*, Vol. 8, No. 5, (2003), pp. 459-465, ISSN 1083-7159

Bedin, V., Adam, R.L., de Sá, B.C., Landman, G., & Metze, K. (2010). Fractal dimension of chromatin is an independent prognostic factor for survival in melanoma. *BMC Cancer*, Vol. 10, (Jun 2010), pp. 260, ISSN 1471-2407

Bedogni, B., Welford, S.M., Cassarino, D.S., Nickoloff, B.J., Giaccia, A.J., & Powell, MB. (2005). The hypoxic microenvironment of the skin contributes to Akt-mediated melanocyte transformation. *Cancer Cell*, Vol. 8, No. 6, (Dec 2005), pp. 443-454, ISSN 1535-6108

Bemis, L.T., Chen, R., Amato, C.M., Classen, E.H., Robinson, S.E., Coffey, D.G., Erickson, P.F., Shellman, Y.G., & Robinson, W.A. (2008). MicroRNA-137 targets microphthalmia-associated transcription factor in melanoma cell lines. *Cancer Res*, Vol. 68, No. 5, (Mar 2008), pp. 1362-1368, ISSN 0008-5472.

Ben-Tabou de-Leon, S., & Davidson, E.H. (2009). Modeling the dynamics of transcriptional gene regulatory networks for animal development. *Dev Biol*, Vol. 325, No. 2, (Jan 2009), pp. 317-328, ISSN 0012-1606

Bennett, DC. (2003). Human melanocyte senescence and melanoma susceptibility genes. *Oncogene*, Vol. 22, No. 20, (May 2003), pp. 3063-3069, ISSN 0950-9232

Bennett, D.C. (2008). How to make a melanoma: what do we know of the primary clonal events? *Pigment Cell Melanoma Res*, Vol. 21, No. 1, (Feb 2008), pp. 27-38, ISSN 1755-1471

Berger, S.L. (2002) Histone modifications in transcriptional regulation. *Curr Opin Genet Dev*, Vol. 12, No. 2, (Apr 2002), pp.142-148, ISSN 0959-437X

Bird, A. (2002). DNA methylation patterns and epigenetic memory. *Genes Dev*, Vol. 16, No. 1, (Jan 2002), pp. 6-21, ISSN 0890-9369

Bird, A.P., & Wolffe, A.P. (1999). Methylation-induced repression--belts, braces, and chromatin. *Cell*, Vol. 99, No. 5, (Nov 1999), pp. 451-454, ISSN 0092-8674

Bittner, M., Meltzer, P., Chen, Y., Jiang, Y., Seftor, E., Hendrix, M., Radmacher, M., Simon, R., Yakhini, Z., Ben-Dor, A., Sampas, N., Dougherty, E., Wang, E., Marincola, F., Gooden, C., Lueders, J., Glatfelter, A., Pollock, P., Carpten, J., Gillanders, E., Leja, D., Dietrich, K., Beaudry, C., Berens, M., Alberts, D., & Sondak, V. (2000). Molecular classification of cutaneous malignant melanoma by gene expression profiling. *Nature*, Vol. 406, No. 6795, (Aug 2000), pp 536-540, ISSN 0028-0836

Board, R.E., Knight, L., Greystoke, A., Blackhall, FH., Hughes, A., Dive, C., & Ranson M. (2008). DNA methylation in circulating tumour DNA as a biomarker for cancer. *Biomark Insights*, Vol. 2. (Jan 2008), pp. 307-319, ISSN 1177-2719

Bode, A.M., & Dong, Z. (2003). Mitogen-activated protein kinase activation in UV-induced signal transduction. *Sci STKE*, Vol. 2003, No. 167, (Jan 2003), pp. RE2, ISSN 1945-0877

Bonazzi, V.F., Irwin, D., & Hayward, N.K. (2009). Identification of candidate tumor suppressor genes inactivated by promoter methylation in melanoma. *Genes Chromosomes Cancer*, Vol. 48, No. 1, (Jan 2009), pp 10-21, ISSN 1045-2257

Bonello, S., Zähringer, C., BelAiba, R.S., Djordjevic, T., Hess, J., Michiels, C., Kietzmann, T., & Görlach, A. (2007). Reactive oxygen species activate the HIF-1alpha promoter via a functional NFkappaB site. *Arterioscler Thromb Vasc Biol*, Vol. 27, No. 4 (Apr 2007), pp. 755-761, ISSN 1049-8834

Campoli, M.R., Chang, C.C., Kageshita, T., Wang, X., McCarthy, J.B., & Ferrone, S. (2004). Human high molecular weight-melanoma-associated antigen (HMW-MAA): a melanoma cell surface chondroitin sulfate proteoglycan (MSCP) with biological and clinical significance. *Crit Rev Immunol*, Vol. 24, No. 4, (2004), pp. 267-296, ISSN 1040-8401

Cancer facts and figures; 2009. Atlanta Cancer Society, Available from: http://www.cancer.org/acs/groups/content/@nho/documents/document/f861009final90809pdf.pdf

Chen, X., Wang, J., Shen, H., Lu, J., Li, C., Hu, D.N., Dong, X.D., Yan, D., & Tu, L. (2011). Epigenetics, MicroRNAs, and Carcinogenesis: Functional Role of MicroRNA-137 in Uveal Melanoma. *Invest Ophthalmol Vis Sci*, Vol. 52, No. 3, (Mar 2011), pp. 1193-1199, ISSN 0146-0404

Chin, L., Merlino, G., & DePinho, R.A. (1998) Malignant melanoma: modern black plague and genetic black box. *Genes Dev*, Vol. 12, No. 22, (Nov 1998), pp 3467-3481, ISSN 0890-936

Chin, L., Garraway, L.A., & Fisher, D.E. (2006). Malignant melanoma: genetics and therapeutics in the genomic era. *Genes Dev*, Vol. 20, No. 16, (Aug 2006), pp. 2149-2182, ISSN 0890-9369

Cloos, P.A., Christensen, J., Agger, K., Maiolica, A., Rappsilber, J., Antal, T., Hansen, K.H., & Helin, K. (2006). The putative oncogene GASC1 demethylates tri- and dimethylated lysine 9 on histone H3. *Nature*, Vol. 442, No. 7100, (Jul 2006), pp. 307-311, ISSN 0028-0836

de la Serna, I.L., Ohkawa, Y., Higashi, C., Dutta, C., Osias, J., Kommajosyula, N., Tachibana, T., & Imbalzano, A.N. (2006). The microphthalmia-associated transcription factor requires SWI/SNF enzymes to activate melanocyte-specific genes. *J Biol Chem*, Vol. 281, No. 29, (Jul 2006), pp. 20233-20241, ISSN 0021-9258

Dhomen, N., Reis-Filho, J.S., da Rocha Dias, S., Hayward, R., Savage, K., Delmas, V., Larue, L., Pritchard, C., & Marais, R. (2009). Oncogenic Braf induces melanocyte senescence and melanoma in mice. *Cancer Cell*, Vol. 15, No. 4, (Apr 2009), pp. 294-303, ISSN 1535-6108

Dong, Z., & Bode, A.M. (2006). The role of histone H3 phosphorylation (Ser10 and Ser28) in cell growth and cell transformation. *Mol Carcinog*, Vol. 45, No. 6, (Jun 2006), pp. 416-421, ISSN 0899-1987

Dynek, J.N., Chan, S.M., Liu, J., Zha, J., Fairbrother, W.J., & Vucic, D. (2008). Microphthalmia-associated transcription factor is a critical transcriptional regulator of melanoma inhibitor of apoptosis in melanomas. *Cancer Res*, Vol. 68, No. 9, (May 2008), pp. 3124-3132, ISSN 0008-5472

Eden, A., Gaudet, F., Waghmare, A., & Jaenisch, R. (2003). Chromosomal instability and tumors promoted by DNA hypomethylation. *Science*, Vol. 300, No. 5618, (Apr 2003), pp. 455, ISSN 0036-8075

Erler, J.T., Bennewith, K.L., Nicolau, M., Dornhöfer, N., Kong, C., Le, Q.T., Chi, J.T., Jeffrey, S.S., & Giaccia, A.J. (2006). Lysyl oxidase is essential for hypoxia-induced metastasis. *Nature*, Vol. 440, No. 7088, (Apr 2006), pp. 1222-1226, ISSN 0028-0836

Facchetti, F., Previdi, S., Ballarini, M., Minucci, S., Perego, P., & La Porta, C.A. (2004). Modulation of pro- and anti-apoptotic factors in human melanoma cells exposed to histone deacetylase inhibitors. *Apoptosis*, Vol. 9, No. 5, (Sep 2004), pp. 573-582, ISSN 1360-8185

Fecher, L.A., Cummings, S.D., Keefe, M.J., & Alani, R.M. (2007). Toward a molecular classification of melanoma. *J Clin Oncol*, Vo. 25, No 12, (Apr 2007), pp. 1606-1620, ISSN 0732-183X

Feinberg, A.P., Cui, H., & Ohlsson, R. (2002). DNA methylation and genomic imprinting: insights from cancer into epigenetic mechanisms. *Semin Cancer Biol*, Vol. 12, No. 5, (Oct 2002), pp. 389-398, ISSN 0008-5472

Fischle, W., Wang, Y., & Allis, C.D. (2003). Histone and chromatin cross-talk. *Curr Opin Cell Biol*, Vol. 15, No. 2, (Apr 2003), pp. 172-183, ISSN 1369-5266.

Flaherty, K.T., Puzanov, I., Kim, K.B., Ribas, A., McArthur, G.A., Sosman, J.A., O'Dwyer, P.J., Lee, R.J., Grippo, J.F., Nolop, K., & Chapman, P.B. (2010). Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med*, Vol. 363, No. 9, (Aug 2010), pp. 809-819, ISSN 0028-4793

Forsythe, J.A., Jiang, B.H., Iyer, N.V., Agani, F., Leung, S.W., Koos, R.D., & Semenza, G.L. (1996). Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol Cell Biol*, Vol. 16, No. 9, (Sep 1996), pp. 4604-4613, ISSN 0270-7306

Fried, L., & Arbiser, J.L. (2008). The reactive oxygen-driven tumor: relevance to melanoma. *Pigment Cell Melanoma Res*, Vol. 21, No. 2, (Apr 2008), pp. 117-122, ISSN 1755-148X

Fruehauf, J.P., & Trapp, V. (2008). Reactive oxygen species: an Achilles' heel of melanoma? *Expert Rev Anticancer Ther*, Vol. 8, No. 11, (Nov 2008), pp. 1751-1757, ISSN 1473-7140

Galabova-Kovacs, G., Matzen, D., Piazzolla, D., Meissl, K., Plyushch, T., Chen, A.P., Silva, A., & Baccarini, M. (2006). Essential role of B-Raf in ERK activation during extraembryonic development. *Proc Natl Acad Sci U S A*, Vol. 103, No. 5, (Jan 2006), pp. 1325-1330, ISSN 0027-8424

Gallagher, W.M., Bergin, O.E., Rafferty, M., Kelly, Z.D., Nolan, I.M., Fox, E.J., Culhane, A.C., McArdle, L., Fraga, M.F., Hughes, L., Currid, C.A., O'Mahony, F., Byrne, A., Murphy, A.A., Moss, C., McDonnell, S., Stallings, R.L., Plumb, J.A., Esteller, M., Brown, R., Dervan, P.A., & Easty, D.J. (2005). Multiple markers for melanoma progression regulated by DNA methylation: insights from transcriptomic studies. *Carcinogenesis*, Vol. 26, No. 11, (Nov 2005), pp.1856-1867, 0143-3334

Garraway, L.A., Widlund, H.R., Rubin, M.A., Getz, G., Berger, A.J., Ramaswamy, S., Beroukhim, R., Milner, D.A., Granter, S.R., Du, J., Lee, C., Wagner, S.N., Li, C., Golub, T.R., Rimm, D.L., Meyerson, M.L., Fisher, D.E., & Sellers, W.R. (2005). Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. *Nature*, Vol. 436, No. 7047, (Jul 2005), pp. 117-122, ISSN 0028-0836

Garraway, L.A. (2010). From cancer genomics to personalized oncology. *Program and abstracts of the American Association for Cancer Research 101st Annual Meeting*, Plenary PL02, Washington, DC, USA, April 17-21, 2010

Gidanian, S., Mentelle, M., Meyskens, F.L. Jr, & Farmer, P.J. (2008). Melanosomal damage in normal human melanocytes induced by UVB and metal uptake--a basis for the pro-oxidant state of melanoma. *Photochem Photobiol*, Vol. 84, No. 3, (May-Jun 2008), pp. 556-564, ISSN 1751-1097

Gill, G. (2004). SUMO and ubiquitin in the nucleus: different functions, similar mechanisms? *Genes Dev*, Vol. 18, No. 17, (Sep 2004), pp. 2046-2059, ISSN 0890-9369

Girdwood, D., Bumpass, D., Vaughan, O.A., Thain, A., Anderson, L.A., Snowden, A.W., Garcia-Wilson, E., Perkins, N.D., & Hay, R.T. P300 transcriptional repression is mediated by SUMO modification. *Mol Cell*, Vol. 11, No. 4, (Apr 2003), pp. 1043-1054, ISSN 1097-2765.

Gómez Román, J.J., Garay, G.O., Saenz, P., Escuredo, K., Sanz Ibayondo, C., Gutkind, S., Junquera, C., Simón, L., Martínez, A., Fernández Luna, J.L., & Val-Bernal, J.F. (2008). Plexin B1 is downregulated in renal cell carcinomas and modulates cell growth. *Transl Res*, Vol. 151, No. 3, (Mar 2008), pp. 134-140, ISSN 1931-5244

Goutzanis, L., Papadogeorgakis, N., Pavlopoulos, P.M., Katti, K., Petsinis, V., Plochoras, I., Pantelidaki, C., Kavantzas, N., Patsouris, E., & Alexandridis, C. (2008). Nuclear fractal dimension as a prognostic factor in oral squamous cell carcinoma. *Oral Oncol*, Vol. 44, No. 4, (Apr 2008), pp. 345-353, ISSN 1368-8375

Guil, S., & Esteller, M. (2009). DNA methylomes, histone codes and miRNAs: tying it all together. *Int J Biochem Cell Biol*, Vol. 41, No. 1, (Jan 2009), pp. 87-95, ISSN 1357-2725

Handolias, D., Hamilton, A.L., Salemi, R., Tan, A., Moodie, K., Kerr, L., Dobrovic, A., & McArthur, G.A. (2010). Clinical responses observed with imatinib or sorafenib in melanoma patients expressing mutations in KIT. *Br J Cancer*, Vol. 102, No. 8, (Apr 2010), pp. 1219-1223, ISSN 0007-0920

Harbst, K., Staaf, J., Måsbäck, A., Olsson, H., Ingvar, C., Vallon-Christersson, J., Ringnér, M., Borg, A., & Jönsson, G. (2010). Multiple metastases from cutaneous malignant melanoma patients may display heterogeneous genomic and epigenomic patterns. *Melanoma Res*, Vol. 20, No. 5, (Oct 2010), pp. 381-391, ISSN 1473-5636

Hashimshony, T., Zhang, J., Keshet, I., Bustin, M., & Cedar, H. (2003). The role of DNA methylation in setting up chromatin structure during development. *Nat Genet*, Vol. 34, No. 2, (Jun 2003), pp 187-92, ISSN 1061-4036

Hauschild, A., Agarwala, S.S., Trefzer, U., Hogg, D., Robert, C., Hersey, P., Eggermont, A., Grabbe, S., Gonzalez, R., Gille, J., Peschel, C., Schadendorf, D., Garbe, C., O'Day, S., Daud,A., White, J.M., Xia, C., Patel, K., Kirkwood, J.M., & Keilholz, U. (2009). Results of a phase III, randomized, placebo-controlled study of sorafenib in

combination with carboplatin and paclitaxel as second-line treatment in patients with unresectable stage III or stage IV melanoma. *J Clin Oncol*, Vol. 27, No. 17, (Jun 2009), pp. 2823-2830, ISSN 0732-183X

He, Z., Cho, Y.Y., Ma, W.Y., Choi, H.S., Bode, A.M., & Dong, Z. (2004). Regulation of ultraviolet B-induced phosphorylation of histone H3 at serine 10 by Fyn kinase. *J Biol Chem*, Vol. 280, No. 4, (Jan 2005), pp. 2446-2454, ISSN 0021-9258

Herman, J.G. (1999). Hypermethylation of tumor suppressor genes in cancer. *Semin Cancer Biol*, Vol. 9, No. 5, (Oct 1999), pp. 359-367, ISSN 1044-579X

Hocker, T.L., Singh, M.K., & Tsao, H. (2008). Melanoma genetics and therapeutic approaches in the 21st century: moving from the benchside to the bedside. *J Invest Dermatol*, Vol. 128, No. 11, (Nov 2008), pp. 2575-2595, ISSN 0022-202X

Hoege, C., Pfander, B., Moldovan, G.L., Pyrowolakis, G., & Jentsch, S. (2002). RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. *Nature*, Vol. 419, No. 6903, (Sep 2002), pp. 135-141, ISSN 0028-0836

Hoek, K.S., Schlegel, N.C., Brafford, P., Sucker, A., Ugurel, S, Kumar, R., Weber, B.L., Nathanson, K.L., Phillips, D.J., Herlyn, M., Schadendorf, D., & Dummer, R. (2006). Metastatic potential of melanomas defined by specific gene expression profiles with no BRAF signature. *Pigment Cell Res*, Vol. 19, No. 4, (Aug 2006), pp. 290-302, ISSN 0893-5785

Hoon, D.S., Spugnardi, M., Kuo, C., Huang, SK., Morton, D.L., & Taback, B. (2004). Profiling epigenetic inactivation of tumor suppressor genes in tumors and plasma from cutaneous melanoma patients. *Oncogene*, Vol. 23, No. 22, (May 2004), pp. 4014-4022, ISSN 0950-9232

Horner, M.J., Ries, L.A.G., Krapcho, M., Neyman, N., Aminou, R., Howlader, N., Altekruse, S.F., Feuer, E.J., Huang, L., Mariotto, A., Miller, B.A., Lewis, D.R., Eisner, M.P., Stinchcomb, D.G., & Edwards, B.K. (January, 7, 2011). In: *SEER Cancer Statistics Review, 1975-2006. Bethesda, MD: National Cancer Institute*, Available from: http://www.seer.cancer.gov/csr/1975_2007/index.html

Houben, R., Becker, J.C., Kappel, A., Terheyden, P., Bröcker, E.B., Goetz, R., & Rapp, U.R. (2004). Constitutive activation of the Ras-Raf signaling pathway in metastatic melanoma is associated with poor prognosis. *J Carcinog*, Vol. 3, No. 1, (Mar 2004). pp. 6, ISSN 0974-6773

Howell, P.M. Jr, Liu, S., Ren, S., Behlen, C., Fodstad, O., & Riker, A.I. (2009). Epigenetics in human melanoma. *Cancer Control*, Vol. 16, No. 3, (Jul 2009), pp. 200-218, ISSN 1073-2748

Jenuwein, T., & Allis, C.D. (2001). Translating the histone code. *Science*, Vol. 293, No. 5532, (Aug 2001), pp. 1074-1080, ISSN 0036-8075

Jiang B.H., Jiang G., Zheng J.Z., Lu Z., Hunter T., & Vogt, P.K. (2001). Phosphatidylinositol 3-kinase signaling controls levels of hypoxia-inducible factor 1. *Cell Growth Differ*, Vol. 12, No. 7, (Jul 2001), pp. 363-369, ISSN 1044-9523

Jones, P.A., & Baylin, S.B. (2002). The fundamental role of epigenetic events in cancer. *Nat Rev Genet*, Vol. 3, No. 6, (Jun 2002), pp. 415-428, ISSN 1471-0056

Joosse, A., De Vries, E., van Eijck, C.H., Eggermont, A.M., Nijsten, T., & Coebergh, J.W. (2010). Reactive oxygen species and melanoma: an explanation for gender

differences in survival? *Pigment Cell Melanoma Res*, Vol. 23, No. 3, (Jun 2010), pp. 352-364, ISSN 1755-1471

Kageshita, T., Nakamura, T., Yamada, M., Kuriya, N., Arao, T., & Ferrone, S. (1991). Differential expression of melanoma associated antigens in acral lentiginous melanoma and in nodular melanoma lesions. *Cancer Res*, Vol. 51, No. 6, (Mar 1991), pp. 1726-1732, ISSN 0008-5472

Karpf, A.R., Lasek, A.W., Ririe, T.O., Hanks, A.N., Grossman, D., & Jones, D.A. (2004). Limited gene activation in tumor and normal epithelial cells treated with the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine. *Mol Pharmacol*, 65, No. 1 (Jan 2004), pp. 18-27, ISSN 0026-895X

Keenen, B., Qi, H., Saladi, S.V., Yeung, M., & de la Serna, I.L. (2010). Heterogeneous SWI/SNF chromatin remodeling complexes promote expression of microphthalmia-associated transcription factor target genes in melanoma. *Oncogene*, Vol. 29, NO. 1, (Jan 2010), pp. 81-92, ISSN 0950-9232

Kietzmann, T., & Görlach, A. (2005). Reactive oxygen species in the control of hypoxia-inducible factor-mediated gene expression. *Semin Cell Dev Biol*, Vol. 16, No. 4-5, (Aug-Oct 2005), pp. 474-486, ISSN 1084-9521

Kim, E.S., Herbst, R.S., Lee, J.J., Blumenschein, G.R. Jr, Tsao, A., Alden, C.M., Tang, X., Liu, S., Stewart, D.J., Heymach, J.V., Tran, H.T., Hicks, M.E., Erasmus, J. Jr, Gupta, S., Powis, G., Lippman, S.M., Wistuba, I.I., & Hong, W.K. (2010). The BATTLE trial (Biomarker-integrated Approaches of Targeted Therapy for Lung Cancer Elimination): personalizing therapy for lung cancer. *Program and abstracts of the American Association for Cancer Research 101st Annual Meeting*, LB-01, Washington, DC, USA, April 17-21, 2010

Kim, G.D., Ni, J., Kelesoglu, N., Roberts, R.J., & Pradhan, S. (2002). Co-operation and communication between the human maintenance and de novo DNA (cytosine-5) methyltransferases. *EMBO J*, Vol. 21, NO. 15, (Aug 2002), pp. 4183-4195, ISSN 0261-4189

Kietzmann, T.,& Görlach, A. (2005). Reactive oxygen species in the control of hypoxia-inducible factor-mediated gene expression. *Semin Cell Dev Biol*, Vol. 16, No. 4-5, (Aug-Ott 2005, pp. 474-486, ISSN 1084-9521

Klose, R.J., & Bird, A.P. (2000). Genomic DNA methylation: the mark and its mediators. *Trends Biochem Sci*, Vol. 31, No, 2, (Feb 2006), pp. 89-97, ISSN 0968-0004

Kuphal, S., Poser, I., Jobin, C., Hellerbrand, C., & Bosserhoff, A.K. (2004). Loss of E-cadherin leads to upregulation of NFkappaB activity in malignant melanoma. *Oncogene*, Vol. 23, No. 52, (Nov 2004), pp. 8509-8519, ISSN 0950-9232

Kyrgidis, A., Tzellos, T.G., & Triaridis, S. (2010). Melanoma: Stem cells, sun exposure and hallmarks for carcinogenesis, molecular concepts and future clinical implications. *J Carcinog*, Vol. 9, (Apr 2010), pp. 3, ISSN 0143-3334

Kuphal, S., Winklmeier, A., Warnecke, C., & Bosserhoff, A.K. (2010). Constitutive HIF-1 activity in malignant melanoma. *Eur J Cancer*, Vol. 46, No. 6, (Apr 2010), pp. 1159-1169, ISSN 0959-8049

La Vecchia, C., Bosetti, C., Lucchini, F., Bertuccio, P., Negri, E., Boyle, P., & Levi, F. (2010). Cancer mortality in Europe, 2000-2004, and an overview of trends since 1975. *Ann Oncol*, Vol. 21, No. 6, (Jun 2010), pp. 1323-1360, ISSN 0923-7534

Levati, L., Alvino, E., Pagani, E., Arcelli, D., Caporaso, P., Bondanza, S., Di Leva, G., Ferracin, M., Volinia, S., Bonmassar, E., Croce, C.M., & D'Atri, S. (2009). Altered expression of selected microRNAs in melanoma: antiproliferative and proapoptotic activity of miRNA-155. *Int J Oncol*, Vol. 35, No. 2, (Aug 2009), pp. 393-400, ISSN 1019-6439

Li, B., Carey, M., & Workman, J.L. (2007). The role of chromatin during transcription. *Cell*, Vol. 128, No. 4, (Feb 2007), pp. 707-719, ISSN 0092-8674

Li, G., Kalabis, J., Xu, X., Meier, F., Oka, M., Bogenrieder, T., & Herlyn, M. (2003). Reciprocal regulation of MelCAM and AKT in human melanoma. *Oncogene*, Vol. 22, No. 44, (Oct 2003), pp. 6891-6899, ISSN 0950-9232

Li, N., & Karin, M. (1999). Is NF-kappaB the sensor of oxidative stress? *FASEB J*, Vol. 13, No. 10, (Jul 1999), pp. 1137-1143, ISSN 0892-6638

Lieberman-Aiden, E., van Berkum, N.L., Williams, L., Imakaev, M., Ragoczy, T., Telling, A., Amit, I., Lajoie, B.R., Sabo, P.J., Dorschner, M.O., Sandstrom, R., Bernstein, B., Bender, M.A., Groudine, M., Gnirke, A., Stamatoyannopoulos, J., Mirny, L.A., Lander, E.S., & Dekker, J. (2009). Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science*, Vol. 326, No. 5950, (Oct 2009), pp 289-293, ISSN 0036-8075

Lin, H., Wong, R.P., Martinka, M., & Li, G. (2010). BRG1 expression is increased in human cutaneous melanoma. *Br J Dermatol*, Vol. 163, No. 3, (Sep 2010), pp. 502-510, ISSN 0007-0963

Lin, W.M., Baker, A.C., Beroukhim, R., Winckler, W., Feng, W., Marmion, J.M., Laine, E., Greulich, H., Tseng, H., Gates, C., Hodi, F.S., Dranoff, G., Sellers, W.R., Thomas, R.K., Meyerson, M., Golub, T.R., Dummer, R., Herlyn, M., Getz, G., & Garraway, L.A. (2008). Modeling genomic diversity and tumor dependency in malignant melanoma. *Cancer Res*, Vol. 68, No. 3, (Feb 2008), pp. 664-673, ISSN 0008-5472

Liu, S., Ren, S., Howell, P., Fodstad, O., & Riker, A.I. (2008). Identification of novel epigenetically modified genes in human melanoma via promoter methylation gene profiling. *Pigment Cell Melanoma Res*, Vol 21., No. 5, (Oct 2008), pp. 545-558, ISSN 1755-148X

Lomas, J., Martin-Duque, P., Pons, M., & Quintanilla, M. (2008). The genetics of malignant melanoma. *Front Biosci*, Vol. 13, (May 2008), pp. 5071-5093, ISSN 1093-9946

Lo Muzio, L., Nocini, P., Mignogna, M.D., Pannone, G., Staibano, S., Procaccini, M., Rubini, C., Fioroni, M., Fanali, S., & Piattelli, A. (2000). Immunocytochemical detection of hMSH2 and hMLH1 expression in oral melanoma. *Anticancer Res*, Vol. 20, No. 2, (Mar-Apr 2000), pp. 741-748, ISSN 0250-7005

Lund, L.P., & Timmins, G.S. (2007). Melanoma, long wavelength ultraviolet and sunscreens: controversies and potential resolutions. *Pharmacol Ther*, Vol. 114, No. 2, (May 2007), pp. 198-207, ISSN 0163-7258

Maddodi, N., & Setaluri, V. (2008). Role of UV in cutaneous melanoma. *Photochem Photobiol*, Vol. 84, No. 2, (Mar-Apr 2008), pp. 528-536, ISSN 0031-8655

Martinez, R., Martin-Subero, J.I., Rohde, V., Kirsch, M., Alaminos, M., Fernandez, A.F., Ropero, S., Schackert, G., & Esteller, M. (2009). A microarray-based DNA methylation study of glioblastoma multiforme. *Epigenetics*, Vol. 4, No. 4, (May 2009), pp. 255-264, ISSN 1559-2294

Mascolo, M., Vecchione, M.L., Ilardi, G., Scalvenzi, M., Molea, G., Di Benedetto, M., Nugnes, L., Siano, M., De Rosa, G., & Staibano, S. (2010). Overexpression of Chromatin Assembly Factor-1/p60 helps to predict the prognosis of melanoma patients. *BMC Cancer*, Vol. 10, (Feb 2010), pp 63, ISSN 1471-2407

Mashiah, A., Wolach, O., Sandbank, J., Uziel, O., Raanani, P., & Lahav, M. (2008). Lymphoma and leukemia cells possess fractal dimensions that correlate with their biological features. *Acta Haematol*, Vol. 199, No. 3, (Apr 2008), pp. 142-150, ISSN 0001-5792

Matsumoto, S., Banine, F., Struve, J., Xing, R., Adams, C., Liu, Y., Metzger, D., Chambon, P., Rao, M.S., & Sherman, L.S. (2005). Brg1 is required for murine neural stem cell maintenance and gliogenesis. *Dev Biol*, Vol. 289, No. 2, (Jan 2006), pp. 372-383, ISSN 0012-1606

Meyle, K.D., & Guldberg, P. (2009). Genetic risk factors for melanoma. *Hum Genet*, Vol. 126, No. 4, (Oct 2009), pp. 499-510, ISSN 0340-6717

Meyskens, F.L. Jr, Farmer, P., & Fruehauf, J.P. (2001). Redox regulation in human melanocytes and melanoma. *Pigment Cell Res*, Vol. 14, No. 3, (Jun 2001), pp. 148-154, ISSN 0893-5785

Michaylira, C.Z., & Nakagawa, H. (2006). Hypoxic microenvironment as a cradle for melanoma development and progression. *Cancer Biol Ther*, Vol. 5, No. 5, (May 2006), pp. 476-479, ISSN 1538-4047

Minet, E., Arnould, T., Michel, G., Roland, I., Mottet, D., Raes, M., Remacle, J., & Michiels, C. (2000). ERK activation upon hypoxia: involvement in HIF-1 activation. *FEBS Lett*, Vol. 468, No. 1, (Feb 2000), pp. 53-58, ISSN 0014-5793

Misteli, T. (2007). Beyond the sequence: cellular organization of genome function. *Cell*, Vol. 128, No. 4, (Feb 2007), pp. 787-800, ISSN 0092-8674

Monsel, G., Ortonne, N., Bagot, M., Bensussan, A., & Dumaz, N. (2010). c-Kit mutants require hypoxia-inducible factor 1alpha to transform melanocytes. *Oncogene*, Vol. 29, No. 2, (Jan 2010), pp. 227-236, ISSN 0950-9232

Montironi, R., Scarpelli, M., Lopez-Beltran, A., Mazzucchelli, R., Alberts, D., Ranger-Moore, J., Bartels, H.G., Hamilton, P.W., Einspahr, J., & Bartels, P.H. (2007). Chromatin phenotype karyometry can predict recurrence in papillary urothelial neoplasms of low malignant potential. *Cell Oncol*, Vol. 29, No. 1, (2007) pp. 47-58, ISSN 1570-5870

Mori, T., Martinez, S.R., O'Day, S.J., Morton, D.L., Umetani, N., Kitago, M., Tanemura, A., Nguyen, S.L., Tran, A.N., Wang, H.J., & Hoon, D.S. (2006). Estrogen receptor-alpha methylation predicts melanoma progression. *Cancer Res*, Vol. 66, No. 13, (Jul 2006), pp. 6692-6698, ISSN 0008-5472

Mueller, D.W., Rehli, M., & Bosserhoff, A.K. (2009). miRNA expression profiling in melanocytes and melanoma cell lines reveals miRNAs associated with formation and progression of malignant melanoma. *J Invest Dermatol*, Vol. 129, No. 7, (Jul 2009), pp. 1740-1751, ISSN 0022-202X

Müller, S., Ledl, A., & Schmidt, D. (2004). SUMO: a regulator of gene expression and genome integrity. *Oncogene*, Vol. 23, No. 11, (Mar 2004), pp. 1998-2008, ISSN 0950-9232

Müller, D.W., & Bosserhoff, A.K. (2008). Integrin beta 3 expression is regulated by let-7a miRNA in malignant melanoma. *Oncogene*, Vol. 27, No. 52, (Nov 2008), pp. 6698-6706, ISSN 0950-9232

Munshi, A., Tanaka, T., Hobbs, M.L., Tucker, S.L., Richon, V.M., & Meyn, R.E. (2006). Vorinostat, a histone deacetylase inhibitor, enhances the response of human tumor cells to ionizing radiation through prolongation of gamma-H2AX foci. *Mol Cancer Ther*, Vol. 5, No. 8, (Aug 2006), pp. 1967-1974, ISSN 1535-7163

Murata, H., Ashida, A., Takata, M., Yamaura, M., Bastian, B.C., & Saida, T. (2007) Establishment of a novel melanoma cell line SMYM-PRGP showing cytogenetic and biological characteristics of the radial growth phase of acral melanomas. *Cancer Sci*, Vol. 98, No. 7, (Jul 2007), pp. 958-963, ISSN 1349-7006

Muthusamy,V., Duraisamy, S., Bradbury, C.M., Hobbs, C., Curley, D.P., Nelson, B., & Bosenberg, M. (2006). Epigenetic silencing of novel tumor suppressors in malignant melanoma. *Cancer Res*, Vol. 66, No. 33, (Dec 2006), pp. 11187-11193, ISSN 0008-5472

Nakayama, K. (2010). Growth and progression of melanoma and non-melanoma skin cancers regulated by ubiquitination. *Pigment Cell Melanoma Res*, Vol. 23, No. 3, (Jun 2010), pp. 338-351, ISSN 1755-1471

Nazarian, R., Shi, H., Wang, Q., Kong, X., Koya, R.C., Lee, H., Chen, Z., Lee, M.K., Attar, N., Sazegar, H., Chodon, T., Nelson. S.F., McArthur, G., Sosman, J.A., Ribas, A., & Lo, R.S. (2010). Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. *Nature*, Vol. 468, No. 7326, (Dec 2010), pp. 973-977, ISSN 0028-0836

Nguyen, T., Kuo, C., Nicholl, M.B., Sim, M.S., Turner, R.R., Morton, D.L., & Hoon, D.S. (2011). Downregulation of microRNA-29c is associated with hypermethylation of tumor-related genes and disease outcome in cutaneous melanoma. *Epigenetics*, Vol. 6, No. 3, (Mar 2011), pp. 388-394, ISSN 1559-2294

Nihal, M., Roelke, C.T., & Wood, G.S. Anti-melanoma effects of vorinostat in combination with polyphenolic antioxidant (-)-epigallocatechin-3-gallate (EGCG). *Pharm Res*, Vol. 27, No. 6, (Jun 2010), pp. 1103-1114, ISSN 0724-8741

Nishikawa, M., & Hashida, M. (2006). Inhibition of tumour metastasis by targeted delivery of antioxidant enzymes. *Expert Opin Drug Deliv*, Vol. 3, No. 3, (May 2006), pp. 355-369, ISSN 1742-5247

Nishikawa, M. (2008). Reactive oxygen species in tumor metastasis. *Cancer Lett*, Vol. 266, No. 1, (Jul 2008), pp. 53-9, ISSN 0304-3835

Noble, I. (2003-04-14). "Human genome finally complete". *BBC News. http://news.bbc.co.uk/1/hi/sci/tech/2940601.stm*. Retrieved 2006-07-22.

Noma, K., Allis, C.D., & Grewal, S.I. (2001). Transitions in distinct histone H3 methylation patterns at the heterochromatin domain boundaries. *Science*, Vol. 293, No. 5532, (Aug 2001), pp. 1150-1155, ISSN 0036-8075

Okano, M., Bell, D.W., Haber, D.A, & Li, E. (1999). DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell*, Vol. 99, No. 3, pp. 247-257, ISSN 0092-8674

Oram, S.W., Liu, X.X., Lee, T.L., Chan, W.Y., & Lau, Y.F. (2006). TSPY potentiates cell proliferation and tumorigenesis by promoting cell cycle progression in HeLa and NIH3T3 cells. *BMC Cancer*, Vol. 6, (Jun 2006), pp. 154, ISSN 1471-2407

Ota, T., Suto, S., Katayama, H., Han, Z.B., Suzuki, F., Maeda, M., Tanino, M., Terada, Y., & Tatsuka, M. (2002). Increased mitotic phosphorylation of histone H3 attributable to AIM-1/Aurora-B overexpression contributes to chromosome number instability. *Cancer Res*, Vol. 62, No. 18, (Sep 2002), pp. 5168-5177, ISSN 0008-5472

Patnaik, M.M., Tefferi, A., & Pardanani, A. (2007). Kit: molecule of interest for the diagnosis and treatment of mastocytosis and other neoplastic disorders. *Curr Cancer Drug Targets*,Vol. 7, No. 5, (Aug 2007), pp. 492-503, ISSN 1568-0096

Pavel, S., van Nieuwpoort, F., van der Meulen, H., Out, C., Pizinger, K., Cetkovská, P., Smit, N.P., & Koerten, H.K. (2004). Disturbed melanin synthesis and chronic oxidative stress in dysplastic naevi. *Eur J Cancer*, Vol. 40, No. 9, (Jun 2004), pp. 1423-1430, ISSN 1359-6349

Piccart-Gebhart, M.J.(2010). The Breast International Group (BIG): building on an accelerated path to tailored adjuvant cancer therapy. *Program and abstracts of the American Association for Cancer Research 101st Annual Meeting*, Plenary PL01, Washington, DC, USA, April 17-21, 2010

Platz, A., Egyhazi, S., Ringborg, U., & Hansson, J. (2008). Human cutaneous melanoma; a review of NRAS and BRAF mutation frequencies in relation to histogenetic subclass and body site. *Mol Oncol*, Vol. 1, No. 4, (Apr 2008), pp. 395-405, ISSN 1574-7891

Pradhan, S., Bacolla, A., Wells, R.D., & Roberts, R.J. (1999). Recombinant human DNA (cytosine-5) methyltransferase. I. Expression, purification, and comparison of de novo and maintenance methylation. *J Biol Chem*, Vol. 274, No. 46, (Nov 1999), pp. 33002-33010, ISSN 0021-9258

Rhee, I., Jair, K.W., Yen, R.W., Lengauer, C., Herman, J.G., Kinzler, K.W., Vogelstein, B., Baylin, S.B., & Schuebel, K.E. (2000). CpG methylation is maintained in human cancer cells lacking DNMT1. *Nature*, Vol. 404, No. 6781, (Apr 2000), pp. 1003-1007, ISSN 0028-0836

Rius, J., Guma, M., Schachtrup, C., Akassoglou, K., Zinkernagel, A.S., Nizet, V., Johnson, R.S., Haddad, G.G., & Karin, M. (2008). NF-kappaB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1alpha. *Nature*, Vol. 453, No. 7196, (Jun 2008), pp. 807-811, ISSN 0028-0836

Robertson, K.D. (2005). DNA methylation and human disease. *Nat Rev Genet*, Vol. 6, No. 8, (Aug 2005), pp. 597-610, ISSN 1471-0056

Rody, A., Karn, T., Ruckhäberle, E., Hanker, L., Metzler, D., Müller, V., Solbach, C., Ahr, A., Gätje, R., Holtrich, U., & Kaufmann, M. (2009). Loss of Plexin B1 is highly prognostic in low proliferating ER positive breast cancers--results of a large scale microarray analysis. *Eur J Cancer*, Vol. 45, No. 3, (Feb 2009), pp. 405-413, ISSN 0959-8049

Romano, M.F., Avellino, R., Petrella, A., Bisogni, R., Romano, S., & Venuta, S. (2004). Rapamycin inhibits doxorubicin-induced NF-kappaB/Rel nuclear activity and enhances the apoptosis of melanoma cells. *Eur J Cancer*, Vol. 40, No. 18, (Dec 2004), pp.2829-2836, ISSN 0014-2964

Romano, S., D'Angelillo, A., Pacelli, R., Staibano, S., De Luna, E., Bisogni, R., Eskelinen, E.L., Mascolo, M., Calì, G., Arra, C., & Romano, M.F. (2010). Role of FK506-binding protein 51 in the control of apoptosis of irradiated melanoma cells. *Cell Death Differ*, Vol. 17, No. 1; (Jan 2010), pp. 145-157, ISSN 1350-9047

Romano, S., D'Angelillo, A., Staibano, S., Ilardi, G., & Romano, M.F. (2010) FK506-binding protein 51 is a possible novel tumoral marker. *Cell Death Dis*, Vol. 1, No. 7, (Jul 2010), pp. e55, ISSN: 2041-4889Ross, S., Best, J.L., Zon, L.I., & Gill, G. (2002). SUMO-1 modification represses Sp3 transcriptional activation and modulates its subnuclear localization. *Mol Cell*, Vol. 10, No.4, (Oct 2002), pp 831-842, ISSN 1097-2765

Rothhammer, T., & Bosserhoff, A.K. (2007). Epigenetic events in malignant melanoma. *Pigment Cell Res*, Vol. 20, No. 2, (Apr 2007), pp. 92-111, ISSN 0893-5785

Rother, J., & Jones, D. (2009). Molecular markers of tumor progression in melanoma. *Curr Genomics*, Vol. 10, No. 4, (Jun 2009), pp. 231-239, ISSN 1389-2029

Rountree, M.R., Bachman, K.E., Herman, J.G., & Baylin, S.B. (2001). DNA methylation, chromatin inheritance, and cancer. *Oncogene*, Vol. 20, No. 24, (May 2001), pg 3156-3165, ISSN 0950-9232

Rubinfeld, B., Robbins, P., El-Gamil, M., Albert, I., Porfiri, E., & Polakis, P. (1997). Stabilization of beta-catenin by genetic defects in melanoma cell lines. *Science*, Vol. 275, No. 5307, (Mar 1997), pp. 1790-1792, ISSN 0036-8075

Rubinstein, J.C., Sznol, M., Pavlick, A.C., Ariyan, S., Cheng, E., Bacchiocchi, A., Kluger, H.M., Narayan, D., & Halaban, R. (2010).Incidence of the V600K mutation among melanoma patients with BRAF mutations, and potential therapeutic response to the specific BRAF inhibitor PLX4032. *J Transl Med*, Vol. 14, No. 8, (Jul 2010), pp. 67, ISSN 1479-5876

Ryu, B., Kim, D.S., Deluca, A.M., & Alani, R.M. (2007). Comprehensive expression profiling of tumor cell lines identifies molecular signatures of melanoma progression. PLoS One, Vol. 2, No. 7, (Jul 2007), e594, ISSN 1932-6203

Saladi, S.V., Keenen, B., Marathe, H.G., Qi, H., Chin, K.V., & de la Serna, I.L. (2010). Modulation of extracellular matrix/adhesion molecule expression by BRG1 is associated with increased melanoma invasiveness. *Mol Cancer*, Vol. 9, (Oct 2010), pp. 280; ISSN 1476-4598

Sander, C.S., Hamm, F., Elsner, P., & Thiele, J.J. (2003). Oxidative stress in malignant melanoma and non-melanoma skin cancer. *Br J Dermatol*, Vol. 148, No. 5, (May 2003), pp. 913-922, ISSN 0007-0963

Santos-Rosa, H., & Caldas, C. (2005). Chromatin modifier enzymes, the histone code and cancer. *Eur J Cancer*, Vol. 41, No. 16, (Nov 2005), pp. 2381-2402, ISSN 1359-6349

Satzger, I., Mattern, A., Kuettler, U., Weinspach, D., Voelker, B., Kapp, A., & Gutzmer, R. (2010). MicroRNA-15b represents an independent prognostic parameter and is correlated with tumor cell proliferation and apoptosis in malignant melanoma. *Int J Cancer*, Vol. 126, No. 11, (Jun 2010), pp 2553-2562, ISSN 0020-7136

Schinke, C., Mo, Y., Yu, Y., Amiri, K., Sosman, J., Greally, J., & Verma, A. (2010). Aberrant DNA methylation in malignant melanoma. *Melanoma Res*, Vol. 20, No. 4, (Aug 2010), pp. 253-265, ISSN 0960-8931

Schultz, J., Lorenz, P., Gross, G., Ibrahim, S., & Kunz M. (2008). MicroRNA let-7b targets important cell cycle molecules in malignant melanoma cells and interferes with anchorage-independent growth. *Cell Res*, Vol. 18, No. 5, (May 2008), pp. 549-557, ISSN 1001-0602

Schultz, J., Ibrahim, S.M., Vera, J., & Kunz, M. (2009). 14-3-3sigma gene silencing during melanoma progression and its role in cell cycle control and cellular senescence. *Mol Cancer*, Vol.8, (Jul 2009), pp. 53, ISSN 14764598

Segura, M.F., Hanniford, D., Menendez, S., Reavie, L., Zou, X., Alvarez-Diaz, S., Zakrzewski, J., Blochin, E., Rose, A., Bogunovic, D., Polsky, D., Wei, J., Lee, P., Belitskaya-Levy, I., Bhardwaj, N., Osman, I., & Hernando, E. (2009). Aberrant miR-182 expression promotes melanoma metastasis by repressing FOXO3 and microphthalmia-associated transcription factor. *Proc Natl Acad Sci U S A*, Vol. 106, No. 6, (Feb 2009), pp. 1814-1819, ISSN 0027-8424

Shackleton, M., & Quintana, E. (2010). Progress in understanding melanoma propagation. *Mol Oncol*, Vol. 4, No. 5, (Oct 2010), pp. 451-457, ISSN 1574-7891

Shen, L., Kondo, Y., Guo, Y., Zhang, J., Zhang, L., Ahmed, S., Shu, J., Chen, X., Waterland, R.A., & Issa, J.P. (2007). Genome-wide profiling of DNA methylation reveals a class of normally methylated CpG island promoters. *PLoS Genet*, Vol. 3, No. 10, (Oct 2007), pp 2023-2036, ISSN 1553-7390

Shiama, N. (1997). The p300/CBP family: integrating signals with transcription factors and chromatin. *Trends Cell Biol*, Vol. 7, No. 6, (Jun 1997), pp. 230-236, ISSN 0962-8924

Shiio, Y., & Eisenman, R.N. (2003). Histone sumoylation is associated with transcriptional repression. *Proc Natl Acad Sci U S A*, Vol. 100, No. 23, (Nov 2003), pp. 13225-13230, ISSN 0027-8424

Shinojima, Y., Terui, T., Hara, H., Kimura, M., Igarashi, J., Wang, X., Kawashima, H., Kobayashi, Y., Muroi, S., Hayakawa, S., Esumi, M., Fujiwara, K., Ghosh, S., Yamamoto, T., Held, W., & Nagase, H. (2010). Identification and analysis of an early diagnostic marker for malignant melanoma: ZAR1 intra-genic differential methylation. *J Dermatol Sci*, Vol. 59, No. 2, (Aug 2010), pp. 98-106, ISSN 0923-1811

Siena, S., Sartore-Bianchi, A., Di Nicolantonio, F., Balfour, J., & Bardelli, A. (2009). Biomarkers predicting clinical outcome of epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer. *J Natl Cancer Inst*, Vol. 101, No. 19, (Oct 2009), pp. 1308-1324, ISSN 0027-8874

Sigalotti, L., Fratta, E., Coral, S., Cortini, E., Covre, A., Nicolay, H.J., Anzalone, L., Pezzani, L., Di Giacomo, A.M., Fonsatti, E., Colizzi, F., Altomonte, M., Calabrò, L., & Maio, M. (2007). Epigenetic drugs as pleiotropic agents in cancer treatment: biomolecular aspects and clinical applications. *J Cell Physiol*, Vol. 212, No. 2, (Aug 2007), pp. 330-344, ISSN 0021-9541

Sigalotti, L., Covre, A., Fratta, E., Parisi, G., Colizzi, F., Rizzo, A., Danielli, R., Nicolay, H.J., Coral, S., & Maio, M. (2010). Epigenetics of human cutaneous melanoma: setting the

stage for new therapeutic strategies. *J Transl Med*, Vol. 8, (Jun 2010), pp. 56, ISSN 1479-5876

Sood, P., Krek, A., Zavolan, M., Macino, G., & Rajewsky, N. (2006). Cell-type-specific signatures of microRNAs on target mRNA expression. *Proc Natl Acad Sci U S A*, Vol. 103, No. 8, (Feb 2006), pp 2746-2751, ISSN 0027-8424

Staibano, S., Lo Muzio, L., Pannone, G., Somma, P., Farronato, G., Franco, R., Bambini, F., Serpico, R., & De Rosa, G. (2001). P53 and hMSH2 expression in basal cell carcinomas and malignant melanomas from photoexposed areas of head and neck region. *Int J Oncol*, Vol. 19, No. 3, (Sep 2001), pp. 551-559, ISSN 1019-6439

Stevens, L., McClelland, L., Fricke, A., Williamson, M., Kuo, I., & Scott, G. (2010). Plexin B1 suppresses c-Met in melanoma: a role for plexin B1 as a tumor-suppressor protein through regulation of c-Met. *J Invest Dermatol*, Vol. 130, No. 6, (Jun 2010), pp. 1636-1645, ISSN 0022-202X

Stine, M.J., Wang, C.J., Moriarty, W.F., Ryu, B., Cheong, R., Westra, W.H., Levchenko, A., & Alani, R.M. (2011). Integration of Genotypic and Phenotypic Screening Reveals Molecular Mediators of Melanoma-Stromal Interaction. *Cancer Res*, Vol. 71, No. 7, (Apr 2011), pp 2433-2444, ISSN 0008-5472

Stratton, M.R., Campbell, P.J., & Futreal, P.A. (2009). The cancer genome. *Nature*, Vol. 458, No. 7239, (Apr 2009), pp. 719-724, ISSN 0028-0836

Sun B., Zhang D., Zhang S., Zhang W., Guo H., & Zhao, X. (2007). Hypoxia influences vasculogenic mimicry channel formation and tumor invasion-related protein expression in melanoma. *Cancer Lett*, Vol. 249, No. 2, (May 2007), pp. 188-197, ISSN 0304-3835

Takata, M., Murata, H., & Saida, T. (2010). Molecular pathogenesis of malignant melanoma: a different perspective from the studies of melanocytic nevus and acral melanoma. *Pigment Cell Melanoma Res*, Vol. 23, No. 1, (Feb 2010), pp. 64-71, ISSN 1755-1471

Tellez, C.S., Shen, L., Estécio, M.R., Jelinek, J., Gershenwald, J.E., & Issa, J.P. (2009). CpG island methylation profiling in human melanoma cell lines. *Melanoma Res*, Vol. 19, No. 3, (Jun 2009), 146-155, ISSN 1473-5636

Thomas, N.E., Berwick, M., & Cordeiro-Stone, M. (2006) Could BRAF mutations in melanocytic lesions arise from DNA damage induced by ultraviolet radiation? *J Invest Dermatol*, Vol. 126, No. 8, (Aug 2006), pp. 1693-1696, ISSN 0022-202X

Thompson, J.F., Scolyer, R.A., & Kefford, R.F. (2009). Cutaneous melanoma in the era of molecular profiling. *Lancet*, Vol. 374, No. 9687, (Aug 2009), pp 362-365, ISSN 0140-6736

Tobar, N., Cáceres, M., Santibáñez, J.F., Smith, P.C., & Martínez, J. (2008). RAC1 activity and intracellular ROS modulate the migratory potential of MCF-7 cells through a NADPH oxidase and NFkappaB-dependent mechanism. *Cancer Lett*, Vol. 267, No. 1, (Aug 2008), pp. 125-132, ISSN 0304-3835

Ugurel, S., Houben, R., Schrama, D., Voigt, H., Zapatka, M., Schadendorf, D., Bröcker, E.B., & Becker, J.C. (2007). Microphthalmia-associated transcription factor gene amplification in metastatic melanoma is a prognostic marker for patient survival, but not a predictive marker for chemosensitivity and chemotherapy response. *Clin Cancer Res*, Vol. 13, No. 21, (Nov 2007), pp. 6344-6350, ISSN 1078-0432

Ugurel, S., Utikal, J., & Becker, J.C. Tumor biomarkers in melanoma. *Cancer Control*, Vol. 16, No. 3, (Jul 2009), pp. 219-224, ISSN 1073-2748

Vachtenheim, J., Ondrusová, L., & Borovanský, J. (2010). SWI/SNF chromatin remodeling complex is critical for the expression of microphthalmia-associated transcription factor in melanoma cells. *Biochem Biophys Res Commun*, Vol. 392, No. 3, (Feb 2010), pp. 454-459, ISSN 0006-291X

Verdone, L., Agricola, E., Caserta, M., & Di Mauro, E. (2006). Histone acetylation in gene regulation. *Brief Funct Genomic Proteomic*, Vol. 5, No. 3, (Sep 2006), pp. 209-221, ISSN 2041-2649

Villanueva, J., Vultur, A., Lee, J.T., Somasundaram, R., Fukunaga-Kalabis, M., Cipolla, A.K., Wubbenhorst, B., Xu, X., Gimotty, P.A., Kee, D., Santiago-Walker, A.E., Letrero, R., D'Andrea, K., Pushparajan, A., Hayden, J.E., Brown, K.D., Laquerre, S., McArthur, G.A., Sosman, J.A., Nathanson, K.L., & Herlyn, M. (2010). Acquired resistance to BRAF inhibitors mediated by a RAF kinase switch in melanoma can be overcome by cotargeting MEK and IGF-1R/PI3K. *Cancer Cell*, Vol. 18, No. 6, (Dec 2010), pp. 683-695, ISSN 1535-6108

Wada, K., Maesawa, C., Akasaka, T., & Masuda, T. (2004). Aberrant expression of the maspin gene associated with epigenetic modification in melanoma cells. *J Invest Dermatol*, 2004, Vol. 122, No. 3, (Mar 2004), pp. 805-811, ISSN 0022-202X

Wang, L., McLeod, H.L., & Weinshilboum, R.M. (2011). Genomics and drug response. *N Engl J Med*, Vol. 364, No. 12, (Mar 2011), pp. 1144-1153, ISSN 0028-4793

Wehrle-Haller, B. (2003). The role of Kit-ligand in melanocyte development and epidermal homeostasis. *Pigment Cell Res*, Vol. 16, No. 3, (Jun 2003), pp. 287-296, ISSN 0893-5785

Wenger, R.H. (2002). Cellular adaptation to hypoxia: O2-sensing protein hydroxylases, hypoxia-inducible transcription factors, and O2-regulated gene expression. *FASEB J*, Vol. 16, No. 10 (Aug 2002), pp. 1151-1162, ISSN 0892-6638

Wiesener, M.S., & Maxwell, P.H. (2003). HIF and oxygen sensing; as important to life as the air we breathe? *Ann Med*, Vol. 35, No. 3, (2003), pp. 183-190, ISSN 0785-3890

Wittgen, H.G., & van Kempen, L.C. (2007). Reactive oxygen species in melanoma and its therapeutic implications. *Melanoma Res*, Vol. 17, No. 6, (Dec 2007), pp. 400-409, ISSN 0960-8931

Wu, K., Fuchs, S.Y., Chen, A., Tan, P., Gomez, C., Ronai, Z., & Pan, Z.Q. (2000). The SCF(HOS/beta-TRCP)-ROC1 E3 ubiquitin ligase utilizes two distinct domains within CUL1 for substrate targeting and ubiquitin ligation. *Mol Cell Biol*, Vol.20, No. 4, (Feb 2000), pp.1382-1393, ISSN 0270-7306

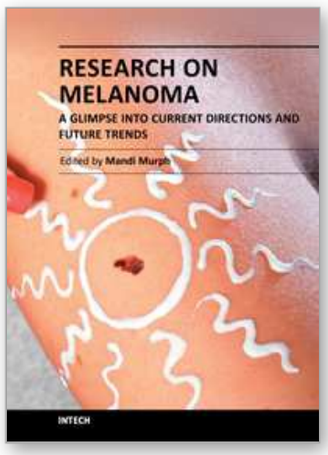
Yang, J., Price, M.A., Li, G.Y., Bar-Eli, M., Salgia, R., Jagedeeswaran, R., Carlson, J.H., Ferrone, S., Turley, E.A., & McCarthy, J.B. (2009). Melanoma proteoglycan modifies gene expression to stimulate tumor cell motility, growth, and epithelial-to-mesenchymal transition. *Cancer Res*, Vol. 69, No. 19, (Oct 2009), pp. 7538-7547, ISSN 0008-5472

Yi, M., Yang, J., Chen, X., Li, J., Li, X., Wang, L., Tan, Y., Xiong, W., Zhou, M., McCarthy, J.B., Li, G., Xiang, B., & Xie, H. (2010). RASSF1A Suppresses melanoma development by modulating apoptosis and cell cycle progression. *J Cell Physiol*, (Dec 2010), ISSN 0021-9541

You, Y., Ma, L., You, M., Li, X., Wang, S., Li, H., Wu, D., Yang, H., & Li, Z.Y. (2010). TSLC1 gene silencing in cutaneous melanoma. *Melanoma Res*, Vol. 20, No.3, (Jun 2010), pp. 179-183, ISSN 1473-5636

Zbytek, B., Carlson, J.A., Granese, J., Ross, J., Mihm, M.C., & Slominski, A. (2008).Current concepts of metastasis in melanoma. *Expert Rev Dermatol*, Vol. 3, No. 5, (Oct 2008), pp. 569-585, ISSN 1746-9872

Zhong, S.P., Ma, W.Y., & Dong, Z. (2000). ERKs and p38 kinases mediate ultraviolet B-induced phosphorylation of histone H3 at serine 10. *J Biol Chem*, Vol. 275, No. 28, (Jul 2000), pp. 20980-20984, ISSN 0021-9258



Research on Melanoma - A Glimpse into Current Directions and Future Trends

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The book Research on Melanoma: A Glimpse into Current Directions and Future Trends, is divided into sections to represent the most cutting-edge topics in melanoma from around the world. The emerging epigenetics of disease, novel therapeutics under development and the molecular signaling aberrations are explained in detail. Since there are a number of areas in which unknowns exist surrounding the complex development of melanoma and its response to therapy, this book illuminates and comprehensively discusses such aspects. It is relevant for teaching the novice researcher who wants to initiate projects in melanoma and the more senior researcher seeking to polish their existing knowledge in this area. Many chapters include visuals and illustrations designed to easily guide the reader through the ideas presented.

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