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An Insight into the Complex Roles of Metallothioneins in Malignant Diseases with Emphasis on (Sub)Isoforms/Isoforms and Epigenetics Phenomena

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Abstract

Metallothioneins (MTs) belong to a group of small cysteine-rich proteins that are ubiquitous throughout all kingdoms. The main function of MTs is scavenging of free radicals and detoxification and homeostating of heavy metals. In humans, 16 genes localized on chromosome 16 have been identified to encode four MT isoforms labelled by numbers (MT-1 – MT-4). MT-2, MT-3 and MT-4 proteins are encoded by a single gene. MT-1 comprises many (sub)isoforms. The known active *MT-1* genes are *MT-1A*, *-1B*, *-1E*, *-1F*, *-1G*, *-1H*, *-1M* and *-1X*. The rest of the *MT-1* genes (*MT-1C*,*-1D*,*-1I*,*-1J* and *-1L*) are pseudogenes. The expression and localization of individual MT (sub)isoforms and pseudogenes vary at intra-cellular level and in individual tissues. Changes in MTs expression are associated with the process of carcinogenesis of various types of human malignancies, or with a more aggressive phenotype and therapeutic resistance. Hence, MT (sub)isoforms profiling status could be utilized for diagnostics and therapy of tumour diseases. This review aims on a comprehensive summary of methods for analysis of MTs at (sub)isoforms levels, their expression in single tumour diseases and strategies how this knowledge can be utilized in anticancer therapy.

Keywords: Metallothioneins, Cancer; Diagnosis; Therapy; Hypermethylation

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Introduction

Metallothioneins (MTs) are a group of low molecular mass, cysteine-rich proteins that have been found in bacteria, plants, invertebrates and vertebrates (Cai, et al., 2014; Ruttkay-Nedecky, et al., 2013). In mammals, number of amino acids in MTs varies from 61 to 68, from which 20 or 21 are 20 cysteines. Due to high thiol groups content, MTs are able to bind 12 monovalent or 7 divalent metal ions and their main functions include maintaining homeostasis of essential metals (Cu and Zn), detoxification of toxic metal ions (Cd) and scavenging free radicals to protect cells against oxidative stress (Klaassen, Liu, & Diwan, 2009). MT-encoding genes are located on chromosome 16 in a cluster and involve 16 identified genes, from which five are pseudogenes. Two pseudogenes and one MT-like gene are located elsewhere, for details see Table 1. In humans, four MT isoforms exist, labelled by numbers (MT-1 – MT-4). MT-2, MT-3 and MT-4 proteins are encoded by a single gene. MT-1 comprises many subtypes encoded by a set of 13 *MT-1* genes. The known active *MT-1* genes are *MT-1A,-1B, -1E, -1F, -1G, -1H,-1M* and *-1X*. The rest of the *MT-1* genes (*MT-1C,-1D,-1I,-1J* and *-1L*) are pseudogenes whose protein product has not been found in humans (Cai, et al., 2014; Romero-Isart & Vasak, 2002). Summary of *MTs* genes, (sub) isoforms, loci and synonyms is shown in Table 1. The most distinctive differences can be found comparing MT-1/MT-2 with MT-3, which contains a conserved acidic hexapeptide insert near the C-terminus in the α-domain, additional threonine residue in β-domain and a unique pair of prolines (-TCPCPS-) near the N-terminus in the β-domain, which are essential for biological activity of MT-3, heavy metals binding properties and association with other proteins, which suggest function diversification in various physiological processes (Bogumil, et al., 1998).

MT-1 and -2 are the most widely expressed in the body, occurring predominantly in tissues of kidney, liver, intestine and pancreas. MT-3 is found mainly in the brain, but it is also expressed ubiquitously in trace amounts. MT-4 can be detected in epithelia and the maternal

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deciduae (Wei, et al., 2008). Other differences can be found at the level of the expression and localization of individual MT (sub)isoforms, which vary at intra-cellular level (cytosol, nucleus, mitochondria and lysosomes) and also in individual tissues (Moleirinho, et al., 2011; Sharma, Rais, Sandhu, Nel, & Ebadi, 2013; Thirumoorthy, et al., 2011).

Questions regarding a purpose of a high number of MT (sub)isoforms and genes arise with increasing knowledge. Even though differences between affinity to zinc and other metals among single isoforms have been found, as well as susceptibility to antioxidants, these differences do not justify such a high number of isoforms, which, as we anticipate, have to have some further biological importance (Schmidt & Hamer, 1986). Mammalian MT-1 and MT-2 are transcriptionally induced conserved proteins essential for metals binding. In most mammalian genomes one copy of *MT-2*, *MT-3*, *MT-4* and multiple copies of *MT-1* are present. Specifically, in human genome, 13 *MT-1* genes are present, from which 5 are pseudogenes. The highest number of *MT-1* copies is found in primate genomes indicating the relatively recent duplication events. The process of gene duplication contributes to phenotypic diversity of living organisms. Novel gene functions arise from mutations altering the sequence of gene product or affecting gene expression. Dynamic changes in tissue expression preference of paralogs with different duplication ages suggest differential contribution of paralogs to specific organ functions. Paralogs are enriched for genes with brain-specific expression and provide evidence for differential forces underlying the preferential emergence of young testis- and liver-specific expressed genes (Guschanski, Warnefors, & Kaessmann, 2017). Phylogenetic analyses show that *MT-1* pseudogenes are derived from functional genes by loss of invariant cysteines and incorporation of aromatic amino acids, and thus accumulation of loss-of-function mutations. The sequence of *MT-4* is highly conserved between humans and mice, but it shows the highest divergence in humans with two structurally disrupting polymorphisms. These polymorphisms reach about 30% frequency in

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African and Asian populations suggesting its non-functionality in some individuals. Some *MT-1* duplicates have cellular specificity and some of them are expressed in epithelium. Taken together with similarities between mouse MT-1 and MT-4 structural and metal binding properties it is possible that the high number of *MT-1* genes compensates and backs-up the loss of *MT-4* gene (Moleirinho, et al., 2011). These findings indicate that the change in expression of single *MT* genes should be changed in the process of carcinogenesis. In the present review we attempt to summarize up-to-date knowledge on the role of MTs (sub)isoforms with special emphasis on their roles in malignant diseases (Fig. 1). Due to the fact that MTs could be helpful as diagnostic and/or prognostic biomarkers in several types of cancers, we also discuss the bioanalytical methods, which enable determination of MTs on (sub)isoform levels. Last but not least, we put our attention on a regulation of MTs by epigenetic processes, whose importance has been evidenced in most of malignancies, and on utilization of regulation of MTs to enhance efficiency of cancer therapy, too.

Methods enabling estimation of MT isoforms and (sub)isoforms

It is clear from the above-mentioned facts that MT exists as a mixture of variable forms. This broad heterogeneity leads to the need for development of powerful separation and bioanalytical techniques that enable the study and understanding of the importance of individual MT (sub)isoforms, however, this is still challenging task. Although there is a high chemical and structural similarity among the isoforms, single MTs are involved in various processes and their expression is dependent on a particular process and tissue. Expression of MTs can be monitored both on nucleic acid level and protein level (Fig. 2), i.e. MT protein presence and its modifications, especially metalation, oxidation, acetylation and methylation (Ogra & Suzuki, 1999; Ryvolova, et al., 2011). However, due to the high structural similarity of MTs, current proteomic methods lack the specificity to distinguish all 11 (sub)isoforms.

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Therefore the most frequent methods for assessment of single MT isoform expression are nucleic-acids based methods, such as *in situ* hybridization, (Q)-RT-PCR and microarrays (Albrecht, et al., 2008; Han, et al., 2013; Krizkova, et al., 2016). These methods allow for detection of *MT* genes polymorphisms, regulation of MT expression both based on MT mRNA synthesis and/or degradation by mechanisms of RNA interference either by determination of mRNA, small RNA or non-coding long RNA presence (J. Yang, et al., 2017). Determination of mRNA does not reflect the amount of MT proteins due to the different mRNA induction and degradation rates as well as RNA-based regulation mechanisms. Thus, determination of both MT protein and mRNA can be useful to obtain complete information (Fig. 2).

For determination of MT proteins, the most of the methods are based on specific chemical properties of MT, especially high thiol groups content and heavy metals content, on which are based Elman's assay, electrochemical and metal-saturation methods, respectively (Bienengraber, Forderkunz, Klein, & Summer, 1995; Dutton, Stephenson, & Klaverkamp, 1993; Krizkova, et al., 2009; Ryvolova, et al., 2011; Savas, Shaw, & Petering, 1993). These methods do not allow distinguishing of specific MT protein isoforms, even though the differences in redox potential and heavy metals affinity have been found. To detect MT isoforms in biological samples, antibody-based methods such as immunohistochemistry, immunocytochemistry, ELISA and western-blotting are most frequently used. Predominantly, the antibodies recognizing MT-1+2 and MT-3 are employed. Due to a high structural similarity between MT-1 and MT-2 isoforms, the development of isoform-specific antibodies is an issue. First the MT-1 and MT-2 isoforms have to be separated or produced by recombinant DNA technology and the obtained antibodies has to be purified from isoform-cross-reactive immunoglobulins (H. M. Chan, Pringle, & Cherian, 1992). Distinguishing of MT-1 (sub)isoforms by using antibodies is even more tricky, due to their high amino acid

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sequence and structural homology, however commercially available anti-MT-1G and anti-MT-1A antibodies have been used for verification of Q-RT-PCR and RNA interference (X. F. Sun, et al., 2016). Antibodies specific to MT-3 most frequently recognize the additional *N*-terminal 6-amino acid-containing domain, which is specific for MT-3 only (Sens, Somji, Garrett, Beall, & Sens, 2001).

Other methods for analysis of MT on a protein level comprise a broad range of spectroscopic methods hyphenated with different separation techniques. Of them, the most predominant are capillary electrophoresis or high-performance liquid chromatography coupled with mass spectroscopy (CZE-MS or HPLC-MS) (Ryvolova, et al., 2011). Mass spectrometry [electrospray ionization (ESI), matrix assisted laser desorption-ionization (MALDI) and inductively coupled plasma (ICP) ionization techniques] represents the most advanced method in metallomics. These techniques provide essential information about protein identity and structure (ESI, MALDI), and elemental composition (ICP). It has to be also noted that some MS-based studies have succeeded in identifying MT (sub)isoforms in human cells either based on tryptic digests (Alvarez, et al., 2012; Shabb, Muhonen, & Mehus, 2017; Wang, et al., 2007), or on unique masses of intact isoforms (Mounicou, et al., 2010; Wang, et al., 2007). Moreover, MALDI imaging allows for studying of proteins distribution in paraffin-embedded tissue slices or cryosections analogical to histology, with the advantage of detection of multiple or unknown analytes without labelling (Arentz, et al., 2017; Norris & Caprioli, 2013; Panderi, et al., 2017; Rodrigo, et al., 2014).

MTs can regulate and be distinctly regulated by a number of biological processes

MTs are involved in regulation of numerous processes, among others, cell proliferation and apoptosis and several aspects of the carcinogenesis or inflammation (Theocharis, Margeli, Klijanienko, & Kouraklis, 2004). Regulative functions of MTs are particularly connected to

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their protein-protein interactions, metal binding and antioxidant properties. The target proteins for interaction belong to transcription and growth factors, cytokines, extracellular matrix degrading enzymes, apoptosis regulators, stress proteins related to oxidative and radiation damage. Transcription factors such as p53 protein, nuclear factor–κB (NF-κB), esophageal cancer-related gene 4 (ECRG4), specificity protein 1 (Sp1), transcription factor IIIA (TFIIIA), estrogen receptor (ER), Gal4 and tramrack (TTK) interact with MTs and change their function. MTs are also source of zinc or copper and therefore activators of various metalloenzymes, for example matrix metalloproteinases (MMP), carbonic anhydrase, alkaline phosphatase (AP), δ-aminolevulinic acid dehydratase, or superoxide dismutase (SOD). Interaction with MTs was documented also at endocytic low-density lipoprotein receptors (LDLRs), especially megalin and lipoprotein receptor related protein 1 (LRP1) (Krizkova, et al., 2012; Zalewska, Trefon, & Milnerowicz, 2014).

Although MTs show increased expression in various tumours (breast, kidney, lung, nasopharynx, ovary, salivary gland, testes, thyroid and bladder cancers, in certain malignancies such as hepatocellular carcinoma, prostate and colorectal cancer, their down-regulation has been evidenced (Gumulec, Raudenska, Adam, Kizek, & Masarik, 2014; S. Takahashi, 2015). Kanda *et al*. have suggested that the mechanisms of MT-1G silencing were related to promoter hypermethylation (Kanda, et al., 2009). Furthermore, representative primary gastric cancer having no expression of MT-3-encoding mRNA demonstrated hypermethylation of the MT-3 intron l CpG island (Deng, et al., 2003). The methylated and unmethylated MT-1 promoters are differentially regulated by DNA methyltransferase and methyl-CpG binding proteins, and the suppression of *MT* promoters by DNA methyltransferase is independent of its enzymatic function (Majumder, et al., 2006). DNA methylation plays an important role in cancer formation by silencing tumour suppressor genes, and thus will be discussed in a separate chapter. Down-regulation of MT synthesis may

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be also connected with mutation of tumour suppressor genes (Cherian, Jayasurya, & Bay, 2003). In *TP53* mutated cell lines MT was not induced and apoptosis was not initiated after the addition of cadmium or copper (Fan & Cherian, 2002). Epigenetic inactivation of *XAF1* tumour suppressor gene is frequently observed in multiple human cancers. Shin et al. presented evidence that XAF1 plays a critical role in cell-fate decisions under heavy metal induced stress conditions through the mutual antagonism with MT-2A. XAF1 is activated as a transcription target of MTF-1 and destabilizes MT-2A through the interaction-directed lysosomal degradation, whereas it is destabilized by MT-2A under cytostatic stress conditions. XAF1-mediated MT-2A inactivation leads to elevation of free intracellular zinc level and up- and down-regulates proteins p53 and XIAP, respectively, to promote apoptosis (Shin, et al., 2017).

MT polymorphisms may increase or decrease the expression efficiency of genes. Highly statistically significant associations were detected between single-nucleotide polymorphisms in core promoter region of MT and Cd, Zn, Cu and Pb levels in prostate cancer tissue (Krzeslak, et al., 2013). MTs are transcriptionally regulated in response to metal ions. A key protein in this process is metal-regulatory factor 1 (MTF1), which binds metal responsive elements located upstream of *MT* genes. Thus, genetic variation in *MTF1* may modulate expression of MT and thereby influence biological management of metals (Adams, et al., 2015).

Connection between epigenetics and MTs regulation human carcinogenesis

Epigenetics, originally defined by C. H. Waddington (Waddington, 1942) as 'the causal interactions between genes and their products, which bring the phenotype into being', involves understanding chromatin structure and its impact on gene functions. The information conveyed by epigenetic alterations plays a crucial role in all DNA-based processes, and thus can have profound influence on the development and maintenance of

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malignant diseases (Dawson & Kouzarides, 2012). As MTs play an important role in many types for solid tumours and leukemias, the significance of epigenetic modifications of *MT* genes in cancer cells merits discussion.

Epigenetic alterations due to DNA methylation processes

Genome-wide analyses have shown that DNA methylation is found in long stretches of chromosome regions containing clusters of contiguous CpG islands or gene families. Hypermethylation of various gene clusters has been reported in many cancer types (Esteller, 2007) (Jadhav, et al., 2015). Several studies, which have performed methylation analyses, identified *de novo* hypermethylation of *MT* promoters associated with consequent MTs silencing. In that way, Jadhav and colleagues revealed that methylation contributes to repression of *MT-1* gene cluster in breast cancer, irrespective of oestrogen receptor (ER) status (Jadhav, et al., 2015). Noteworthy, they also revealed a negative correlation between invasiveness of ERα+ cells (MCF-7) and *MT-1F* and *MT-1M* expression, which thus may play an anti-oncogenic role. Distinct role was identified for MT-3, which is commonly silenced in normal breast tissue and breast-derived cell lines, but can be found in breast cancers tending to poor disease outcome (Gomulkiewicz, et al., 2016; Kmiecik, et al., 2015; Zeisig, Koklic, Wiesner, Fichtner, & Sentjurc, 2007). Interestingly, Somji *et al*. revealed that treatment of non-tumorigenic MCF-10A cells with demethylation agent Decitabine or histone deacetylase inhibitor, Entinostat, restored the expression of MT-3 (Somji, et al., 2010), suggesting its epigenetic regulation. Comparable phenomenon has been also observed in endometrial cancer cells, in which demethylation agent Azacytidine reactivates expression of MT-1E (Tse, et al., 2009). Moreover, it was found that promoter of *MT-1E* was hypermethylated in more than 42% of endometrial carcinoma specimens, but not in normal or hyperplastic endometrial tissue samples.

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It is worth noting that epigenetic regulation can act in a location-specific manner. Peng and co-workers have shown that oesophageal carcinomas display high rate of methylation of CpG of *MT-3* from -372 to -306 from the transcription start site, which was not found in benign specimens (D. F. Peng, et al., 2011). Moreover, they identified a significant correlation between hypermethylation of -127 to -8 CpG sites with advanced tumour stages and lymph node metastases. Deliberately, we do not mention all studies, as they demonstrate similar results (*MT-1F* in colon cancer, *MT-1* in rat hepatoma, *MT-2A* in gastric cancer, *MT-1M* and *MT-1G* in hepatocellular carcinoma or *MT-1G* in thyroid cancer (J. Fu, et al., 2013; Ghoshal, Majumder, Li, Dong, & Jacob, 2000; Ji, et al., 2014; Pan, et al., 2016; Yan, et al., 2012)), but overall, it is evident that hypermethylation of specific regions in CpG islands of selected *MT* genes could be a valuable diagnostic and prognostic marker, warranting further investigation.

One may ask why these events occur. Several factors mechanistically linked with altered methylation have already been identified. During aging a large overlap among hypermethylated genes and tumorigenesis has been identified, and is thus considered as one of the important factors (Klutstein, Nejman, Greenfield, & Cedar, 2016; Kwabi-Addo, et al., 2010; Teschendorff, et al., 2010). Clear molecular links with aberrant DNA methylation were found also for exposures to chemical agents (Hutt, et al., 2005) or inflammatory processes caused by *Helicobacter pylori* or hepatitis B virus (J. Liu, et al., 2006; Niwa, et al., 2010; Su, et al., 2007). Despite that there is still a lack of studies showing the straight links between specific exposures and aberrant methylation of *MTs* genes, which could bring novel insights into carcinogenic processes.

Role of microRNA (miRNA) in post-transcriptional regulation of MTs

MiRNA belong to a class of short (18-25 nucleotides) noncoding RNAs, involved in RNA interference machinery to regulate gene post-transcriptional gene expression (Sato, Tsuchiya,

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Meltzer, & Shimizu, 2011), contributing to physiological and pathophysiological functions including carcinogenesis (Lu, et al., 2005). Although miRNAs were discovered in 1993 (R. C. Lee, Feinbaum, & Ambros, 1993) and till that time it has been intensively investigated, only little is known about relation between miRNA and MTs regulation.

Zhang and co-workers revealed that miR-1246 and miR-1290 are significantly enriched in tumour-initiating cells and play a critical role in regulation of tumour growth and metastasis, particularly through repressing the MT-1G (Zhang, et al., 2016). In gastric cancer, MT-2A was found to be a potential target of miR-23a (An, et al., 2013). A significant inverse correlation between expression of miR-23a and MT-2A was detected in 70% of tumour samples and furthermore, overexpression of miR-23a also greatly reduced both MT-2A protein and mRNA expression levels in gastric epithelial (GES1) cells. Similarly, we have identified negative inverse correlation between miR-376 and MT-2A in malignant prostate cells (22Rv1) and miR-224 and MT-1A in metastatic prostate (PC-3) cells. It is worth noting that miRNAs obviously directly regulates specific genes encoding MTs (sub)isoforms, however further research might be done to fully understand this phenomenon (An, et al., 2013).

Regulation and expression of MTs (sub)isoforms is distinct across various types of malignant diseases

Complex role of MTs in cancer

Numerous immunohistochemical and gene expression studies have demonstrated that changes in MTs expression are associated with the process of carcinogenesis in various types of human malignancies, or are even associated with a more aggressive phenotype and therapeutic resistance, ultimately resulting in a worse prognosis (Gumulec, et al., 2014; Pedersen, Larsen, Stoltenberg, & Penkowa, 2009; Thirumoorthy, et al., 2011). Importantly,

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the change in MT-1/2 protein expression may differ from the change in the expression of single MT isoforms. For instance, MT-1/2 over-expression has been found in cutaneous malignant melanomas in association with poor prognosis (Emri, et al., 2013; Sugita, Yamamoto, & Asahi, 2001; Weinlich, 2009), but it has also been demonstrated that epigenetic down-regulation of MT-1E and MT-1G isoforms might play a role in melanoma progression (Faller, et al., 2010; Koga, et al., 2009). Most likely, some MT isoforms have specific functions in the cells, but the exact mechanisms behind these phenomena remain still unclear. Interestingly, meta-analysis of independent microarray datasets revealed that expression of an inhibitor of apoptosis (*BIRC5*) and certain MT isoforms (*MT-1B, -1E, -1F, -1H, -1X*) clustered in various cancers showing a high interconnection between these genes (Choi, Yu, Yoo, & Kim, 2005). Nevertheless, MT isoform expression pattern in a cancer might reflect the tissue type, differentiation status, proliferative index, the level of inflammation, and perhaps the carcinogenic stimuli and signalling pathways implicated in tumour development (Hanada, Sawamura, Hashimoto, Kida, & Naganuma, 1998; Cherian, et al., 2003). Exploration of changes in expression of particular MT isoforms in various cancers can contribute to better understanding of the process of carcinogenesis and identification of novel therapeutic targets.

To this date numerous studies aiming on MTs in cancer, both in human tumour tissues and cell lines, have been published providing an extensive pool of data. To provide a comprehensive insight into the complicated relation between MTs and cancer, the results showing expression of MTs and their pseudogenes in various tumour tissues are summarized in Table 2, while the overall summary of results obtained from cell cultures *in vitro* are summarized in Tables 3 – 10. As it is obvious from the presented tables, the most data regarding MT (sub)isoforms expression is known for metals exposure, particularly for Cd^{2+} and Zn^{2+}, which are known MT inducers. Noteworthy, induction of *MT* genes is not uniform

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upon metals treatment, as well as it is not within single cell lines even those derived from the same cancer type. The similar trend is seen for other treatments with other metals and cytostatics or inhibitors of cellular processes, natural compounds and/or nanoparticles. Other important fields of studies are focused on regulation of *MT* genes and studies of cancer-related conditions such as chemoresistance, DNA mutations, RNA interference and hypoxia. The most of work for MT-1 (sub)isoforms and MT-4 isoform has been done using nucleic acids-based methods due to the lack of reliable antibodies. On the other hand, expression of MT-2A and MT-3 were also studied using immuno-based assays. Overall, based on the data it should be stated that due to the variability of MTs within various tumour types and conditions, a number of *MT* genes can be identified, whose expression exhibits tumour-related functions, and thus their modulation can reverse the tumour progression. In next sub-chapters we will describe the most notable findings regarding the MTs (sub)isoforms and specific types of malignant diseases.

Prostate cancer

Reduced MT-1/2 protein expression was reported in tissues derived from prostate cancer as compared with benign prostatic hyperplasia (J. D. Lee, Wu, Lu, Yang, & Jeng, 2009), however, in other studies, an increased expression of MT-1/2 and MT-3 has been found in prostate cancer, even it was shown to correlate with the histological grade of neoplasm (Albrecht, et al., 2008; El Sharkawy, Abbas, Badawi, & El Shaer, 2006; Garrett, Sens, et al., 1999). A recent study on 128 patients with prostate cancer demonstrated that high expression of MT-2A protein in cancer cells is associated with a decreased biochemical recurrence-free survival rate (Ma, et al., 2015). The -5 A/G single nucleotide polymorphism (SNP; rs28366003) in core promoter region of MT-2A is able to affect the expression of the *MT-2A* gene in prostatic tissue (Krzeslak, et al., 2013). Compared to homozygous common allele

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carriers, heterozygosity for the G variant is coupled with a significantly increased risk of prostate cancer in a Polish population (Forma, et al., 2012). The expression of MT-2A seems to negatively correlate with Cu, Pb and Ni concentrations in prostate cancer tissues (Krzeslak, et al., 2013). While MT-1A, MT-1E, MT-2A, and MT-3 expressions have been shown in both healthy prostatic tissue and prostate cancer, the expression of *MT-1X* gene could only be detected in normal prostate (Garrett, et al., 2000; Garrett, Sens, et al., 1999). Down-regulation of MT-1G by promoter hypermethylation was demonstrated in 29 (24%) of 121 prostate cancer, 5 (13%) of 39 high-grade prostatic intraepithelial neoplasms, 3 (10%) of 29 benign prostatic hyperplasia, and 0 (0%) of 13 normal prostate tissue samples without significant differences in methylation frequencies or levels (Henrique, et al., 2005). Methylation levels were found to correlate with tumour stage and were more frequent in prostate cancer that spread beyond the prostate capsule (Henrique, et al., 2005). Low expression of MT-1H due to promoter hypermethylation has been described in prostate cancer with poor prognosis (Han, et al., 2013). In a microRNA microarray study on 50 prostate adenocarcinomas with and without perineural invasion, miR-224 has been identified as the most differently expressed microRNA (Prueitt, et al., 2008). This microRNA has been shown to be expressed by perineural cancer cells and to down-regulate MT expression in these cells (Prueitt, et al., 2008). For summary of MTs (sub)isoforms expression studies in human prostate cancer cell lines see Table 3.

Lung cancer

Increased MT-1/2 protein expression has been demonstrated in 62 (89.9%, n=69) non-small cell lung cancer (NSCLC) samples as compared to non-malignant lung tissues (NMLT, n=12) (Werynska, Pula, Muszczynska-Bernhard, Gomulkiewicz, Piotrowska, et al., 2013). Expression of *MT-1B, -1F, -1G, -1H* and *-1X* genes were found to be significantly up-regulated, while *MT-1E* was significantly down-regulated in NSCLC cancer tissues

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(Werynska, Pula, Muszczynska-Bernhard, Gomulkiewicz, Piotrowska, et al., 2013). Higher MT-1B mRNA expression was associated with squamocellular and adenocarcinoma subtype of NSCLC (Werynska, Pula, Muszczynska-Bernhard, Gomulkiewicz, Piotrowska, et al., 2013), where a review of studies on MT expression in human lung cancer cell lines is shown in Table 4. Higher MT-1F mRNA expression was associated with larger primary tumour size, with higher grade of malignancy and poor patients' survival (Werynska, Pula, Muszczynska-Bernhard, Gomulkiewicz, Piotrowska, et al., 2013). In this study, statistically insignificant higher MT-1A mRNA expression was also detected in larger primary tumours, as well as up-regulated MT-2A mRNA that predicted poor prognosis (Werynska, Pula, Muszczynska-Bernhard, Gomulkiewicz, Piotrowska, et al., 2013). In another study, the level of MT-1A, MT-2A, and MTF-1 expression have been shown to be even lower in lung cancer specimens compared to cancer-surrounding tissues (Liang, et al., 2013). Importantly, MT-1X was identified as metastasis related gene in NSCLC cell lines in a very recent study (Y. Liu, et al., 2016). Comparing the expression level of MT-1X in human lung cancer tissues and matched adjacent normal lung tissues, a significant difference could be shown between stages I and IV confirming the prognostic value of *MT-1X* gene expression in clinical settings (Y. Liu, et al., 2016). Five SNPs in the *MT-1* gene region have been found to be associated with increased risk of lung cancer among non-heavy smokers in a Japanese population (rs7196890 showed the strongest association) and the impact of the polymorphisms decreased with the increasing consumption of cigarettes (Nakane, et al., 2015). Expression of MT-3 has also been investigated in lung cancer, and was found to be significantly up-regulated in NSCLC as compared to NMLT (Werynska, Pula, Muszczynska-Bernhard, Gomulkiewicz, Jethon, et al., 2013). In addition, compared with NMLT, higher nuclear, but lower cytoplasmic MT-3 expression could be detected in cancer cells (Werynska, Pula, Muszczynska-Bernhard, Gomulkiewicz, Jethon, et al., 2013). Low cytoplasmic MT-3 expression was associated with

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larger primary tumour size, nevertheless, lower nuclear MT-3 expression was linked with higher tumour grade, and lower MT-3 mRNA expression seemed to be associated with poor patient outcome (Werynska, Pula, Muszczynska-Bernhard, Gomulkiewicz, Jethon, et al., 2013). From the epigenetic point of view, an overall increase in gene promoter methylation has been reported in association with age and environmental exposure in NMLT (Tsou, et al., 2007). Furthermore, an association between methylation status of *MT* genes and gender, histology, asbestos exposure, and lymph node involvement was demonstrated in patients with malignant mesothelioma (Tsou, et al., 2007).

Breast cancer

Disequilibrium in zinc homeostasis and high concentration of zinc in breast cancer tissues has been reported (Chandler, et al., 2016). The increased *MT* gene expression can frequently be detected in breast tumour specimens with predominantly cytoplasmic MT protein expression (see Table 5 for a review of studies on MT expression in human breast cancer cell lines), and it correlates with higher histological grade and significantly lower recurrence-free survival after treatment with adjuvant chemotherapy, but seems to be independent of age, tumour size and oestrogen receptor (OR) status (Yap, et al., 2009). MT-1A, MT-1E, MT-1F, MT-1G, MT-1H, MT-1X and MT-2A but not MT-1B mRNA was detected in invasive ductal breast cancer tissue (IDBC) samples (R. X. Jin, et al., 2002). MT-2A, MT-1E, MT-1F were found to be expressed in both IDBC specimens and their adjacent benign breast tissues, although MT-1F expression seemed to be significantly higher in benign breast tissues compared with the breast cancers; MT-2A was demonstrated as the predominant isoform in both benign and malignant breast tissues (R. X. Jin, Bay, Chow, Tan, & Dheen, 2001; R. X. Jin, et al., 2002). In another study, higher MT-1F mRNA expression was found to be associated with higher histological grade of breast neoplasm (R. X. Jin, Bay, Chow, & Tan, 2001). MT-2A mRNA and MT

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protein expression were found to be in association with cancer cell proliferation (Ki-67 immunolabelling) and histological grade (R. X. Jin, et al., 2002). In case-control studies, SNPs in *MT-2A* (rs1580833 in a German population and rs28366003 in a Polish population) showed a positive association with breast cancer risk (Krzeslak, et al., 2014; Seibold, et al., 2011). In further study, significantly higher MT-1E mRNA expression was detected in OR-negative breast tumour tissues specimens compared to OR-positive ones (R. Jin, Bay, Chow, Tan, & Lin, 2000). Nevertheless, epigenetic repression of *MT-1* gene cluster was also demonstrated in breast cancer (Jadhav, et al., 2015). *In silico* analysis revealed much lower gene expression of this cluster in The Cancer Genome Atlas cohort for OR-positive tumours (Jadhav, et al., 2015). Comparing the methylation of CpG islands in tissues (tumour, healthy breast and blood) from patients with breast cancer revealed that the promoter of MT-1A was methylated above 25% in 18 primary and metastatic tumours, but there was also >10% methylation of healthy breast tissue in 5 samples suggesting that the methylation process for this gene takes place already in normal breast cells (Piotrowski, et al., 2006). Interestingly, metal induced *MT* gene expression also seems to be dependent on epigenetic regulation in breast cancer cells, namely on the histone acetylation status of the gene promoter, which is determined by p53 function (Ostrakhovitch, Olsson, von Hofsten, & Cherian, 2007). In the presence of mutated p53 the expression of *MT-1A* and *MT-2A* is dampened in response to metal, but constitutive MT-3 gene expression is allowed (Ostrakhovitch, Song, & Cherian, 2016). Sens et al. showed that MT-3 over-expression was detected in breast cancer samples, and it was found to be associated with high recurrence rate (Sens, et al., 2001). In another study, however, MT-3 expression has been found to be lower in IDBC specimens compared with non-malignant breast tissues or mastopathies, in addition, the level of MT-3 mRNA was demonstrated to be even lower in breast cancers with lymph node metastasis than in carcinomas without metastasis (Gomulkiewicz, et al., 2016).

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Colorectal cancer

The down-regulation of MT-1/2 expression was revealed in association with colorectal cancer progression, although a relatively high MT content could be detected in colorectal cancers with very poor prognosis (Arriaga, et al., 2012; Janssen, et al., 2000). A review of studies on MT expression in colorectal cancer cell lines is shown in Table 6. Down-regulation of MT-1B (Jansova, et al., 2006), -1E (Arriaga, et al., 2012), -1F (Arriaga, et al., 2012; Jansova, et al., 2006; Yan, et al., 2012), -1G (Arriaga, et al., 2012; Jansova, et al., 2006; Yan, et al., 2012), -1H (Arriaga, et al., 2012; Jansova, et al., 2006), -1M (Arriaga, et al., 2012), -1X (Yan, et al., 2012), and MT-2A (Jansova, et al., 2006; Yan, et al., 2012) has been demonstrated during the transition from normal mucosa to cancer, the less down-regulated expression of MT-1X and MT-2A was thought to support MT protein expression in tumour tissue (Arriaga, et al., 2012). Radiotherapy seems to be able to induce the expression of *MT-1F*, *MT-1X* and *MT-2A* genes in rectal cancer tissue, however, there is no difference in MT-1/2 protein expression levels between the samples obtained before and after radiotherapy (Szelachowska, et al., 2012). Regarding the mechanism of down-regulation of gene expression, promoter hypermethylation of MT-1G (Arriaga, et al., 2012), and loss of heterozygosity at the *MT-1F* locus (Yan, et al., 2012) have been also identified. Noteworthy, in high microsatellite instability colorectal carcinoma tissues MT-1X T20 (3'UTR, T20 mononucleotide repeat of the MT-1X gene) instability can be more frequently detected as compared to microsatellite stable or low microsatellite instability colorectal cancer cases (97.3% sensitivity and 100% specificity) (Morandi, et al., 2012). Serine peptidase inhibitor, Kazal type 1 (SPINK1) that has been shown to contribute to increased cell proliferation, invasion, soft agar colony formation, and therapy resistance in colon adenocarcinoma cell culture through activation of oncogenic signalling pathways, also seemed to be involved in reduced expression of various MT

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isoforms in colon cancer cells as SPINK1 knockdown leads to up-regulation of *MT-1B, -1E, -1G, -1H, -1L, -1M, -1X,* and *MT-2A* genes in these cells (Tiwari, et al., 2015).

Hepatocellular carcinoma

Compared to the adjacent non-malignant liver, significant repression of MT-1G and MT-1M due to promoter hypermethylation has been demonstrated in primary hepatocellular carcinomas (K. Y. Y. Chan, et al., 2006; Kanda, et al., 2009; J. Mao, et al., 2012). A recent study confirmed that low MT-1M expression correlates with high alpha-fetoprotein levels and early (<24 months) tumour recurrence after surgery (Ding & Lu, 2016). Furthermore, the methylation status of MT-1G and MT-1M promoters detected in serum cell free DNA (liquid biopsy) in patients with hepatocellular carcinoma was also shown to be significantly higher than that in patients with chronic hepatitis B or in normal controls (Ji, et al., 2014). In addition, in carcinoma patients associations have been found between serum MT-1M promoter methylation and tumour size, and between simultaneous MT-1G and MT-1M promoter methylation and higher incidence of vascular invasion or metastasis, respectively (Ji, et al., 2014). Association between hypermethylation of the promoter region of MT-1H and liver cancer with poor clinical outcome has also been reported (Han, et al., 2013). Increased activity of DNA methyltransferase 1 (Dnmt1) might be one of the reasons responsible for down-regulation of *MT* gene expression in liver cancer (Takata, et al., 2013). Dnmt1 is a direct target of miR-140, and reduced expression of the microRNA-containing ribonucleoprotein complex component DDX20, which is frequently seen in hepatocellular carcinomas, can lead to the impairment of miR-140 function (Takata, et al., 2013). MT-1M is also a target gene of miR-24-3p that is another significantly up-regulated microRNA in liver cancer tissues as compared with non-tumour liver tissues (Dong, et al., 2016). Furthermore, *MT* gene expression is dependent on DNA binding activity and phosphorylation of

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CCAAT/enhancer binding protein alpha (C/EBPalpha) in liver cells (Datta, et al., 2007). In hepatocellular carcinoma the phosphorylation of C/EBPalpha is decreased due to suppressed activity of glycogen synthase kinase-3, a downstream effector of PI3K/AKT signalling pathway (Datta, et al., 2007). In a hospital-based case-control study it has been revealed that MT-1 rs8052394, rs964372, and rs8052334 A-G-T haplotype can enhance the carcinogenic effect of smoking on liver, and carriers with this haplotype have higher risk for liver cancer development than the control group (A-C-T, the most common haplotype) (Wong, et al., 2013). Decreased expression of *MT-1A, -1E, -1F, -1G, -1H, -1X* genes was demonstrated in intrahepatic cholangiocarcinoma tissue samples as compared with normal liver tissues in patients residing in Northeast Thailand, a region with a high prevalence of liver fluke infection (Subrungruang, et al., 2013). Table 7 summarizes studies on expression of MT in hepatic cancer cell lines.

Head and neck cancer

Significantly higher MT-1/2 expression was observed in oral squamous cell carcinoma tissues comparing with oral leukoplakia or normal epithelial tissue samples (Pontes, et al., 2009). Nevertheless, up-regulation of *MT-1F* gene expression, but down-regulation of *MT-1A*, *MT-1X*, *MT-3* and *MT-4* gene expressions was detected in carcinoma tissue specimens compared with non-neoplastic oral mucosa (Brazao-Silva, et al., 2015). High MT-1X expression in cancer tissues was restricted to non-metastatic cases, but high MT-3 expression was associated with increased risk of lymph node metastasis (Brazao-Silva, et al., 2015). Furthermore, the low level of MT-1G mRNA in carcinoma tissues correlated with poor prognosis (Brazao-Silva, et al., 2015). An SNP analysis revealed that *MT-1* rs11076161 AA, rs964372 CC, and rs7191779 GC genotypes are protective against oral squamous cell carcinomas, whereas *MT-1* rs8052394 A allele is associated with a higher risk to oral cancer

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development (Zavras, Yoon, Chen, Lin, & Yang, 2011). Regarding squamous cell laryngeal cancer, the -5 A/G (rs28366003) SNP in the core promoter region of the *MT-2A* has been shown to be related to the higher cancer risk (Starska, Krzeslak, Forma, Olszewski, Lewy-Trenda, et al., 2014). Moreover, the most carriers of minor allele had a higher stage, increased cancer aggressiveness, as defined by a higher total tumour front grading score and diffuse tumour growth (Starska, Krzeslak, Forma, Olszewski, Lewy-Trenda, et al., 2014). In further study, a significant association between the rs28366003 SNP in the *MT-2A* gene and MT-2A mRNA levels was demonstrated in squamous cell laryngeal cancer and non-cancerous laryngeal mucosa samples, and an inverse relation was shown between MT-2A expression and Cd, Zn and Cu content in tissues (Starska, Krzeslak, Forma, Olszewski, Morawiec-Sztandera, et al., 2014). Table 8 summarizes studies on expression of MT in head and neck cancer cell lines.

Oesophageal cancer

Down-regulation of *MT-1G, -1M,* and *MT-3* gene expressions have been detected in oesophageal squamous cell carcinoma tissue samples as compared with non-malignant oesophageal tissues (Kumar, Chatopadhyay, Raziuddin, & Ralhan, 2007; Y. C. Lee, et al., 2011; Oka, et al., 2009; E. Smith, et al., 2005). Importantly, methylation study on tissue specimens from normal oesophageal mucosae from healthy subjects without carcinogen exposure, normal mucosae from healthy subjects with carcinogen exposure, normal mucosae from cancer patients, and in cancerous mucosae has revealed significantly higher methylation of MT-1M in cancer samples, and in addition, in drinkers and in smokers (Y. C. Lee, et al., 2011; Oka, et al., 2009). Down-regulation of *MT-3* gene expression in oesophageal squamous cell carcinoma seems also to be associated with promoter hypermethylation (E. Smith, et al., 2005). Nevertheless, a study on DNA methylation profiles in the MT-3 promoter region in

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oesophageal adenocarcinomas has revealed that in tumour tissues the CpG nucleotides in two regions (from 2139 to -49 and +296 to +344) were significantly hypermethylated as compared to normal samples, whereas CpG nucleotides from -372 to -306 from the transcription start site were highly methylated in both tumour and normal samples (D. F. Peng, et al., 2011). Furthermore, the DNA hypermethylation from 2127 to 28 CpG sites was found to be associated with advanced cancer and lymph node metastasis (D. F. Peng, et al., 2011). Recently, up-regulation of the expression of a long non-coding RNA, HNF1A-AS1, has been demonstrated in oesophageal adenocarcinomas relative to their corresponding normal oesophageal tissues, and MT-1E was identified as its downstream target (X. Yang, et al., 2014).

Tumours of central nervous system

Gene expression studies on glioblastoma tumour specimens revealed an association between high *MT-1A*, *-1B*, *-1E*, *-1F*, *-1H*, and *MT-3* expression and poor patient survival (Mehrian-Shai, et al., 2015). Moreover, MT-2 protein expression was found to be significantly higher in glioblastoma multiforme tissue samples from the first surgery than in tumour's fragments of the same region but obtained 1 year apart suggesting a dynamic change in *MT* gene expression with progression in this type of cancer (de Aquino, et al., 2016). Very recently, down-regulation of miR-340 and up-regulation of miR-1293 has been shown in glioblastoma multiforme biopsies (Cosset, et al., 2016). Interestingly, several *MT* genes (*MT-1A*, *-1E*, *-1F*, *-1H*, *-1X*, *-2A*) were identified as targets of these microRNAs, but it was emphasised that the induced changes in gene expression is influenced by the cellular micro-environment (Cosset, et al., 2016). Down-regulation of *MT* genes (*MT-1L*, *MT-1G*, *MT-1E*, *MT-1X*, *MT-1B*, *MT-2A*, and *MT-3*) has been demonstrated as a common event at relapse of ependymoma, however, loss or deletion of the *MT* genes cluster could not be demonstrated (Peyre, et al., 2010).

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Methylation of the promoter of *MT-3* gene has been supposed, but could not be proved (Peyre, et al., 2010).

Thyroid cancer

Although the up-regulation of MT expression in follicular thyroid carcinoma has been reported in one study (Back, et al., 2013), several data have been published to demonstrate the down-regulation of MT expression in thyroid cancers (both in papillary and follicular thyroid carcinoma, but to a greater extent in papillary carcinoma) compared to normal thyroid tissue (Ferrario, et al., 2008; J. Fu, et al., 2013; Huang, De La Chapelle, & Pellegata, 2003). It has been demonstrated that promoter methylation contributes to *MT-1G* inactivation in thyroid cancers, even an association between *MT-1G* hypermethylation and lymph node metastasis in papillary thyroid cancer patients has been found (J. Fu, et al., 2013; Huang, et al., 2003). Loss of heterozygosity seems to be a remarkably rare mechanism of loss of *MT-1G* gene function in this cancer (Huang, et al., 2003).

Renal cancer

MT protein expression has been demonstrated in specimens from renal cell carcinoma (RCC) and it was found to be associated with significantly worse prognosis (Nguyen, et al., 2000; Tuzel, Kirkali, Yorukoglu, Mungan, & Sade, 2001). However, down-regulation of MT-1H (Alkamal, et al., 2015; Nguyen, et al., 2000; M. Takahashi, et al., 2001), MT-1G (Alkamal, et al., 2015; M. Takahashi, et al., 2001), MT-2A (Alkamal, et al., 2015), MT-1A, MT-1L and MT-1E (M. Takahashi, et al., 2001) have been shown in RCC. In one study, comparing cancer tissue samples to non-malignant tissues from 11 patients with RCC the same level of MT-1E, MT-1F and MT-1X expression, but up-regulation of MT-2A and down-regulation of MT-1A and MT-1G expression were detected in cancer tissue specimens (Nguyen, et al., 2000).

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Gastric cancer

Lower MT-2A mRNA and protein expression has been detected in gastric cancer tissue samples comparing with the adjacent normal gastric tissues (J. M. Kim, et al., 2005; Pan, Xing, Cui, Li, & Lu, 2013). In addition, loss of MT-2A expression in gastric cancer seems to be associated with down-regulation of I kappa B-alpha expression, diffuse- and intestinal-type histological subtypes, higher grade, and an advanced clinical stage (Pan, Huang, et al., 2013; Pan, Xing, et al., 2013). MT-2A is a potential target of miR-23a, and comparing gastric cancer tissue specimens to matched normal tissues an increase in miR-23a expression has been detected and an inverse correlation was found between miR-23a and MT-2A expression (An, et al., 2013). Nevertheless, expression of MT-2A can be induced by chemotherapy, and high MT-2A expression in gastric cancer tissue is associated with better response to chemotherapy and prolonged patient survival as compared to those with low MT-2A expression (Pan, et al., 2016). Furthermore, it seems to be possible to induce the up-regulation of MT-2A expression by inhibition of histone deacetylase activity in gastric cancer cells (Pan, et al., 2016). Down-regulation of *MT-3* gene expression by hypermethylation has also been found in gastric cancers, particularly in p53-negative cases (Deng, et al., 2003).

Bladder cancer

MT-1/2 protein over-expression has been demonstrated in bladder cancer tissues, whereas MT-1/2 expression could not be detected in non-malignant bladder specimens (Somji, Sens, Lamm, Garrett, & Sens, 2001). In bladder cancer patients a high MT expression in tumour tissues was linked to shorter tumour-specific survival, and increased recurrence rates (Hinkel, Schmidtchen, Palisaar, Noldus, & Pannek, 2008). Expression of mRNA for the *MT-2A* and *MT-1X* genes could be shown in both normal and cancerous bladder tissues, the expression of

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MT-1E was found to be variable, while expression of MT-1X proved to be up-regulated in cancer as compared to the level of MT-1X mRNA in normal bladder tissue (Somji, et al., 2001). In another cohort of patients with bladder cancer the expression of MT-1E has been found to be associated with higher cancer stage (Wu, Siadaty, Berens, Hampton, & Theodorescu, 2008). Using loss of function analysis, the same research group demonstrated that MT-1E expression contributes to cancer cell migration (Wu, et al., 2008). MT-3 protein expression seems to occur frequently in carcinoma in situ as well as in low- and high-grade urothelial cancer (Somji, et al., 2011; Zhou, et al., 2006). In contrast, *MT-3* gene is silenced in non-transformed urothelial cells by a mechanism involving histone modification of the *MT-3* promoter (Somji, et al., 2011).

Endometrium cancer

Loss of MT expression in association with copy number changes has been found to be an early event in development of uterine corpus endometrial carcinoma, and it was found to be associated with poorer prognosis (Delaney & Stupack, 2016). Down-regulation of *MT-1E* gene expression due to promoter hypermethylation could be demonstrated in carcinoma tissue samples, particularly with low OR-alpha expression, as compared with normal endometrial tissues or hyperplasias (Tse, et al., 2009).

Ovarian cancer

Down-regulation of *MT-1L*, *-1X*, and *MT-2A* gene expression could be revealed in ovarian tissues reflective of low malignant potential/early cancer onset and possible pre-malignant stages (Mougeot, et al., 2006). However, the absence of MT protein expression in ovarian cancer samples correlated with improved progression-free survival in patients treated with adjuvant platinum-based chemotherapy (Woolston, et al., 2010).

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Pancreatic cancer

High MT protein expression was detected in pancreas adenocarcinoma tissues compared with pancreatic serous cystadenoma or healthy pancreatic tissue samples (Sliwinska-Mosson, Milnerowicz, Rabczynski, & Milnerowicz, 2009).

Sarcoma and other mesenchymal tumours

Up-regulation of *MT-1B, -1E, -1G, -1H, -1L, -1X*, and *MT-2A* gene expression was found in osteosarcoma tissue samples compared with bone biopsies of non-malignant lesions, and three MT isoforms (*MT-1E, -1H* and *MT-1X*) were among the 10 most highly up-regulated genes in the osteosarcoma transcriptome (Endo-Munoz, Cumming, Sommerville, Dickinson, & Saunders, 2010). An association between MT-1F, -1H, -1X, and MT-2A over-expression in tumour specimens and high metastasis risk has also been observed in patients with high-grade soft tissue sarcoma (Skubitz, Francis, Skubitz, Luo, & Nilbert, 2012). As mentioned above, the down-regulation of MT-2A expression is a frequent finding in gastric cancer tissues compared to adjacent normal tissue samples (J. M. Kim, et al., 2005; Pan, Xing, et al., 2013). Interestingly, comparing MT-2A expression in tissue specimens of gastrointestinal stromal tumour (GIST) located in the stomach with that in early gastric carcinomas, significantly lower MT-2A mRNA expression and nuclear MT protein expression were found in GIST samples (Soo, et al., 2011).

Haematological malignancies

Up-regulation of *MT* gene expression has been demonstrated in diffuse large B-cell lymphoma (DLBCL) with poor prognosis, including activated B-cell and type-3 DLBCL (Poulsen, et al., 2006). In contrast, low to undetectable MT expression has been found in

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germinal center DLBCL (Poulsen, et al., 2006). Down-regulation of *MT-3* gene expression due to promoter methylation has been detected in paediatric acute myeloid leukaemia samples (Y. F. Tao, et al., 2014). Table 9 summarizes studies on expression of MT in human haematological cancer cell lines.

Melanoma and non-melanoma skin cancers

MT-1/2 over-expression has been found in cutaneous malignant melanomas in association with poor prognosis (Emri, et al., 2013; Sugita, et al., 2001; Weinlich, 2009). Over-expression of cancer-testis antigen 16 (CT16, PAGE5), a positive regulator of MT-2A has been demonstrated in melanoma metastasis (Nylund, et al., 2012). Nevertheless, *MT-1E* gene promoter methylation could be revealed in 1 of 17 (6%) of the benign naevi, in 16 of 43 (37%) primary melanoma tumours and in 6 of 13 (46%) melanoma metastases (Faller, et al., 2010). Higher incidence of promoter methylation of *MT-1G* was also demonstrated in melanomas compared with normal melanocytes and nevi (Koga, et al., 2009). Ectopic over-expression of MT-1E has been demonstrated to increase the sensitivity of melanoma cells to cisplatin-induced apoptosis (Faller, et al., 2010). Low MT-3 protein expression has been demonstrated in normal skin epidermis (Pula, et al., 2015; Slusser, et al., 2015). Significantly higher MT-1/2 and MT-3 expression was noted in actinic keratosis and cutaneous squamous cell cancer, as compared with normal skin epidermis, whereas very low levels of MT-3 expression were found in basal cell cancer (Pula, et al., 2015; Slusser, et al., 2015; Zamirska, Matusiak, Dziegiel, Szybejko-Machaj, & Szepietowski, 2012). Table 10 summarizes of MTs (sub)isoforms expression studies in other human cancer cell lines.

Possibilities of using the MTs regulation in cancer therapy

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Above chapter gives clear evidence that due to their roles and altered expressions in tumours MTs could be targeted to enhance the efficiency of anticancer therapy (Lai, Yip, & Bay, 2011). Noteworthy, pretreatment with MT inducers can improve chemotherapy tolerance by decreasing the toxic effects of cytostatics on non-target organs (Heger, et al., 2016). On the other hand, this action can result in significant increase of chemoresistance of cancer cells. Thus, specific knowledge on particular roles of MTs has to be obtained. SiRNA silencing of MTs was already published in (Lai, et al., 2010; Tarapore, Shu, Guo, & Ho, 2011), where Tarapore *et al*. used phage Phi29 Motor pRNA as a vehicle to carry siRNA specifically targeted to MT-2A mRNA in ovarian cancers (Tarapore, et al., 2011). Lai *et al*. (Lai, et al., 2010) reported that silencing of *MT-2A* gene by siRNA induces entosis in MCF-7 breast cancer cells. Targeting of a unique mRNA molecule using antisense approaches, based on sequence specificity of double-stranded nucleic acid interactions should, in theory, allow for design of drugs with high specificity for intended targets. Antisense-induced degradation or inhibition of translation of a target mRNA is potentially capable of inhibiting the expression of any target protein (Jason, Koropatnick, & Berg, 2004). Downregulation of MTs by antisense RNA/DNA is known to inhibit growth of various types of tumour cells. Using this strategy it is possible to inhibit the growth and metastases of breast cancer cells (AbdelMageed & Agrawal, 1997), leukemia P388 cells, Ehrlich carcinoma, sarcoma 180 (Takeda, et al., 1997) and nasopharyngeal cancer cells (O. J. K. Tan, Bay, & Chow, 2005). Antisense MT mRNA may also induce sensitivity of the cancer cells to cytostatic, either heavy metal-based (Kennette, Collins, Zalups, & Koropatnick, 2005) or others, such as anthracyclines (Wulfing, et al., 2007; Yap, et al., 2009) and kinase inhibitors (X. F. Sun, et al., 2016).

Cisplatin resistance was inhibited in mouse melanoma cell line by RNA interference using reducible oligo-peptoplex (J. H. Lee, et al., 2015). In human cell lines the decrease in basal

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MT expression by antisense MT mRNA caused increasing of tumour cells sensitivity to cisplatin (Kennette, et al., 2005). Use of sorafenib, a tyrosine kinase inhibitor, leads to a survival benefit in patients with advanced HCC, but its use is hampered by drug resistance. Targeting *MT-1G* enhances the anticancer activity of sorafenib *in vivo*, where suppression of *MT-1G* expression increased sorafenib sensitivity and negative regulation of ferroptosis in Huh7 and HepG2 cells (X. F. Sun, et al., 2016).

Another potential role of MT in cancer therapy is its protective action during chemotherapy (Volm, 1998). Overall, cells with developed resistance to heavy metal-based cytostatics have often increased expression of MTs (Bredel, 2001; Chao, 1996; Naito, Yokomizo, & Koga, 1999; Perez, 1998; Scanlon, Kashanisabet, Tone, & Funato, 1991). Targeting the MTs with antisense RNA/DNA for reversal of multidrug resistance was successfully proposed (Gosland, Lum, Schimmelpfennig, Baker, & Doukas, 1996), and could be considered as pivotal part of personalized cancer therapy.

Although the use of these approaches demonstrates very promising results, we anticipate that further detailed insights into the complex kingdom of MTs may bring higher therapeutic efficiency. For instance, antisense-based therapy can be targeted to multiplex targets, not only one specific sub-isoform. This can enable for possible multiplication of therapeutic effects, however a lot of experiments is still required to accelerate these applications.

Conclusions and future outlooks

MTs are crucial biological molecules with a wide range of roles. Particularly, in cancer management, the detailed knowledge of changes in MTs expression on sub-isoforms levels allows for a proposal of systems for silencing or restoring their expression with the aim to modulate the efficiency of the treatment protocol and to enhance the patient's outcome. It is worth noting that recent literature shows that the accurate classification of expression pattern

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of MTs could be also helpful to enhance the diagnostic possibilities and patient's stratification for personalized treatment. Despite fast advances in the field of analytical chemistry, the proper identification of MTs on a protein level is still complicated. Anyway, we believe that such methods will allow for exact understanding of expression of certain subisoforms. This progress will accelerate the description of the biological roles of certain MTs, which are indisputably pivotal for a number of pathophysiological processes.

Conflict of Interests

The authors declare no conflict of interests.

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Captions for Figures

Figure 1

Knowledge of MTs different expression and regulation in tumour diseases is usable for their treatments.

Figure 2

Overview of methods for determination of MTs expression with respect to features important in research of tumour diseases. For more information to single methods see (Haq, Mahoney, & Koropatnick, 2003; Krizkova, et al., 2016; Ryvolova, et al., 2011)

Figure 1

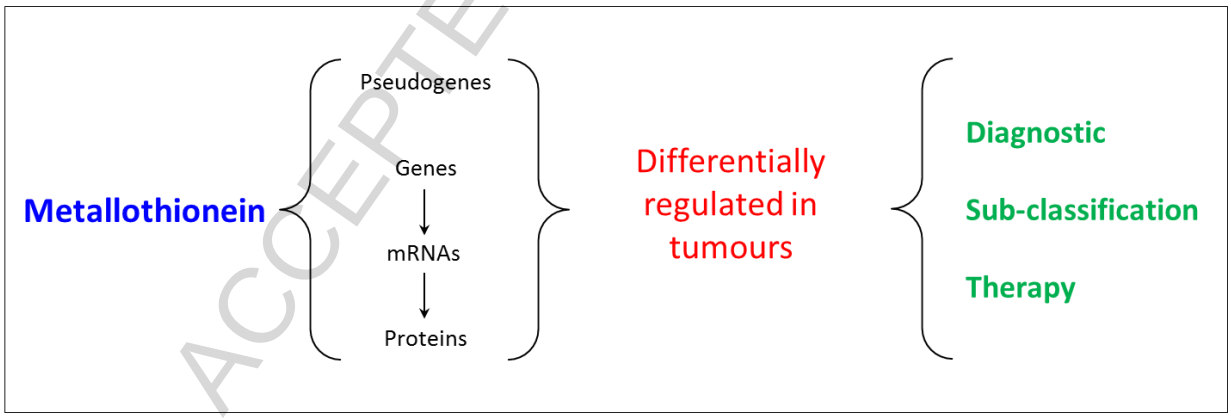

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Figure 2

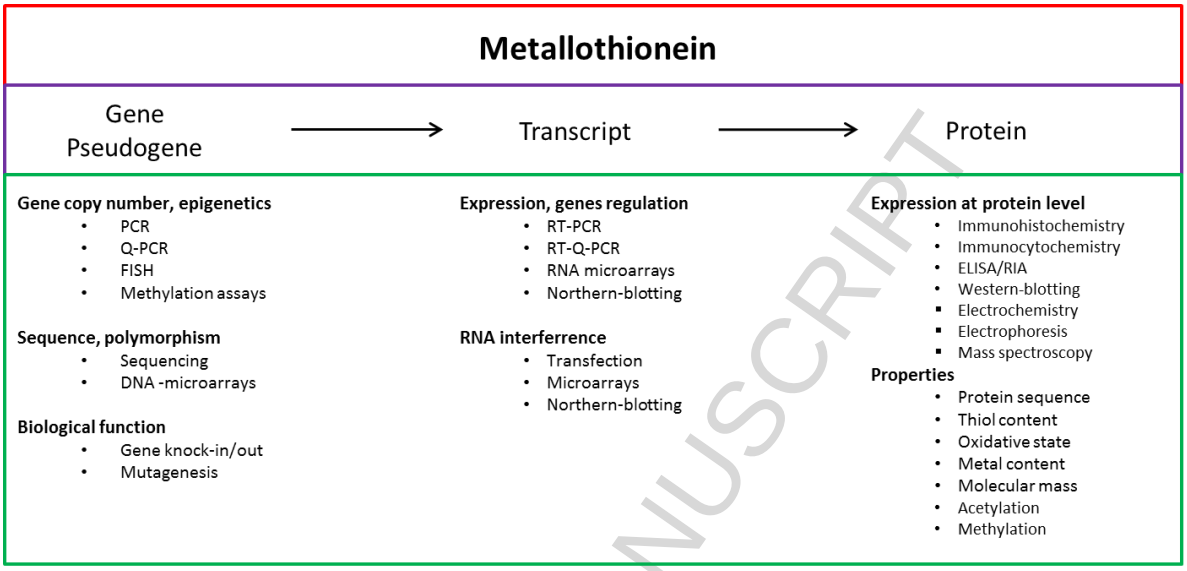

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Table 1. Overview of human MT classification

MT	Isoform	(Sub)isoform	Gene symbol	Gene name	Previous Symbols	Synonyms	Locus
MT	1	A	*MT-1A*	metallothionein 1A	MT1, MT1S		16q13
		B	*MT-1B*	metallothionein 1B	MT1, MT1Q		16q13
		E	*MT-1E*	metallothionein 1E	MT1	MTD	16q13
		F	*MT-1F*	metallothionein 1F	MT1		16q13
		G	*MT-1G*	metallothionein 1G	MT1	MT1K	16q13
		H	*MT-1H*	metallothionein 1H	MT1		16q13
		1HL1	*MT-1HL1*	metallothionein 1H like 1	MT1P2		1q43
		M	*MT-1M*	metallothionein 1M	MT1, MT1K		16q13
		X	*MT-1X*	metallothionein 1X	MT1	MT-1l	16q13
			MT-1CP	metallothionein 1C, pseudogene			16q13
			MT-1DP	metallothionein 1D, pseudogene		MTM	16q13
			MT-1IP	metallothionein 1I, pseudogene	MT1, MT1I	MTE	16q13
			MT-1JP	metallothionein 1J, pseudogene	MT1, MT1NP, MT1J	MTB	16q13
			MT-1L	metallothionein 1L, pseudogene	MT1	MTF, MT1R	16q13
			MT-1P1	metallothionein 1 pseudogene 1		bA435O5.3	9q22.32
			MT-1P3	metallothionein 1 pseudogene 3	C20orf127, MTL4	dJ614O4.6	20q11.22
MT	2	A	*MT-2A*	metallothionein 2A	MT2		16q13
MT	3		*MT-3*	metallothionein 3		GIF	16q13
MT	4		*MT4*	metallothionein 4		MTIV	16q13

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Table 2. Summary of MTs (sub)isoforms expression studies in human tumours. Up- and down regulation is related to surrounding non-tumour tissues, if not mentioned otherwise.

Diagnosis	Gene	Tissue sample	Observation	Citation
Prostate cancer	*MT-1F*	Perineural-invasive CaP	downregulation	(Prueitt, et al., 2008)
	MT-1G	CaP	hypermethylation	(Henrique, et al., 2005)
	MT-1H	CaP	hypermethylation	(Han, et al., 2013)
	MT-1M	Perineural-invasive CaP	downregulation	(Prueitt, et al., 2008)
	MT-1X	Advanced CaP	downregulation	(Garrett, et al., 2000)
Gastric cancer	*MT-1A*	cisPt-resistant gastric cancer	expression	(Suganuma, et al., 2003)
	MT-1B	cisPt-resistant gastric cancer	expression	(Suganuma, et al., 2003)
	MT-1E	cisPt-resistant gastric cancer	expression	(Suganuma, et al., 2003)
	MT-1F	cisPt-resistant gastric cancer	expression	(Suganuma, et al., 2003)
	MT-1G	cisPt-resistant gastric cancer	upregulation	(Suganuma, et al., 2003)
	MT-1JP	Gastric cancer	downregulation	(J. Yang, et al., 2017)
	MT-1M	Gastric cancer	downregulation	(J. Yang, et al., 2017)
	MT-2A	Poor prognosis gastric cancer Docetaxel-responding gastric cancer	downregulation upregulation	(Pan, Huang, et al., 2013; Pan, Xing, et al., 2013) (Pan, et al., 2016)
	MT-3	cisPt resistant gastric cancer Gastric cancer	expression hypermethylation	(Suganuma, et al., 2003) (Deng, et al., 2003)
	MT4	cisPt resistant gastric cancer	expression	(Suganuma, et al., 2003)
Thyroid cancer	*MT-1E*	thyroid cancer	downregulation	(Ferrario, et al., 2008)
	MT-1G	thyroid cancer	hypermethylation downregulation, modulation of PI3K/Akt pathway	(Huang, et al., 2003) (J. Fu, et al., 2013) (Ferrario, et al., 2008)
	MT-1X	thyroid cancer	downregulation	(Ferrario, et al., 2008)
	MT-2A	thyroid cancer	downregulation	(Ferrario, et al., 2008)
Sarcoma	*MT-1B*	osteosarcoma	upregulation	(Endo-Munoz, et al., 2010)
	MT-1E	osteosarcoma	upregulation	(Endo-Munoz, et al., 2010)
	MT-1F	soft tissue sarcoma	upregulation	(Skubitz, et al., 2012)
	MT-1G	osteosarcoma	upregulation	(Endo-Munoz, et al., 2010)
	MT-1H	soft tissue sarcoma osteosarcoma	upregulation upregulation	(Skubitz, et al., 2012) (Endo-Munoz,

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				et al., 2010)
	MT-1L	osteosarcoma	upregulation	(Endo-Munoz, et al., 2010)
	MT-1X	soft tissue sarcoma	upregulation	(Skubitz, et al., 2012)
	MT-2A	soft tissue sarcoma osteosarcoma	upregulation upregulation	(Skubitz, et al., 2012) (Endo-Munoz, et al., 2010)
Breast cancer	*MT-1A*	breast cancer breast cancer	hypermethylation downregulation	(Piotrowski, et al., 2006) (Tai, et al., 2003)
	MT-1B	breast cancer	no expression	(Tai, et al., 2003)
	MT-1E	breast cancer oestrogen negative breast cancer breast cancer	downregulation in tumour area expression dependent on invasivity downregulation	(R. X. Jin, Bay, Chow, Tan, et al., 2001) (R. Jin, et al., 2000) (Tai, et al., 2003)
	MT-1F	breast cancer Different grades breast cancer tissues breast cancer	downregulation in tumour area expression correlation with grade downregulation	(R. X. Jin, Bay, Chow, Tan, et al., 2001) (R. X. Jin, Bay, Chow, & Tan, 2001) (Tai, et al., 2003)
	MT-1G	breast cancer	downregulation	(Tai, et al., 2003)
	MT-1H	breast cancer	downregulation	(Tai, et al., 2003)
	MT-1JP	breast cancer	hypermethylation	(Piotrowski, et al., 2006)
	MT-1X	breast cancer	downregulation	(Tai, et al., 2003)
	MT-2A	breast cancer breast cancer	downregulation in tumour area expression	(R. X. Jin, Bay, Chow, Tan, et al., 2001) (Tai, et al., 2003)
	MT-3	breast cancer with poor prognosis	upregulation	(Sens, et al., 2001)
Lung cancer	*MT-1A*	lung cancer malignant mesothelioma	downregulation hypermethylation	(Liang, et al., 2013) (Tsou, et al., 2007)
	MT-1B	poor outcome NSLC	upregulation	(Werynska, Pula, Muszczynska-Bernhard, Gomulkiewicz, Piotrowska, et al., 2013)
	MT-1E	poor outcome NSLC lung cancer	downregulation downregulation	(Werynska, Pula, Muszczynska-Bernhard, Gomulkiewicz, Piotrowska, et al., 2013) (Liang, et al., 2013)

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	MT-1F	bad prognosis LLC poor outcome NSLC	upregulation upregulation	(da Motta, De Bastiani, Stapenhorst, & Klamt, 2015) (Werynska, Pula, Muszczynska-Bernhard, Gomulkiewicz, Piotrowska, et al., 2013)
	MT-1G	bad prognosis LLC poor outcome NSLC lung cancer	upregulation upregulation downregulation	(da Motta, et al., 2015) (Werynska, Pula, Muszczynska-Bernhard, Gomulkiewicz, Piotrowska, et al., 2013) (Liang, et al., 2013)
	MT-1H	poor outcome NSLC	upregulation	(Werynska, Pula, Muszczynska-Bernhard, Gomulkiewicz, Piotrowska, et al., 2013)
	MT-1M	bad prognosis LLC	upregulation	(da Motta, et al., 2015)
	MT-1X	bad prognosis LLC poor outcome NSLC	upregulation upregulation	(da Motta, et al., 2015) (Werynska, Pula, Muszczynska-Bernhard, Gomulkiewicz, Piotrowska, et al., 2013)
	MT-2A	lung cancer malignant mesothelioma	downregulation hypermethylation	(Liang, et al., 2013) (Tsou, et al., 2007)
	MT-3	lung tissue from patients exposed to sulfur mustard malignant NSLC lung cancer	downregulation nuclear downregulation downregulation	(Tahmasbpour, Ghanei, Qazvini, Vahedi, & Panahi, 2016) (Werynska, Pula, Muszczynska-Bernhard, Gomulkiewicz, Jethon, et al., 2013) (Liang, et al., 2013)
	MT4	lung cancer	downregulation	(Liang, et al., 2013)
Ovarian cancer	*MT-1L*	low malignant potential/early cancer onset	downregulated	(Mougeot, et al., 2006)
	MT-1X	low malignant potential/early cancer onset	downregulation	(Mougeot, et al., 2006)
	MT-2A	low malignant potential/early cancer onset	downregulation	(Mougeot, et al., 2006)

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Melanoma and non-melanoma skin cancers	*MT-1E*	Melanoma	hypermethylation, cisPt sensitivity	(Faller, et al., 2010)
	MT-3	actinic keratosis basal cell carcinoma SCC Melanoma and SCC BCC	upregulation downregulation upregulation moderate to intense expression low to moderate expression	(Pula, et al., 2015) (Pula, et al., 2015) (Pula, et al., 2015) (Slusser, et al., 2015) (Slusser, et al., 2015)
Renal cancer	*MT-1A*	RCC	downregulation	(Nguyen, et al., 2000; M. Takahashi, et al., 2001)
	MT-1E	RCC	downregulation	(M. Takahashi, et al., 2001)
	MT-1G	RCC	downregulation	(Alkamal, et al., 2015; Nguyen, et al., 2000; M. Takahashi, et al., 2001)
	MT-1H	RCC	downregulation	(Alkamal, et al., 2015; M. Takahashi, et al., 2001)
	MT-1L	RCC	downregulation	(M. Takahashi, et al., 2001)
	MT-2A	RCC RCC	downregulation upregulation	(Alkamal, et al., 2015) (Nguyen, et al., 2000)
	MT-3	APA	upregulation	(Felizola, et al., 2014)
Hepatocellular carcinoma	*MT-1A*	ICC HCC	downregulation downregulation	(Subrungruang, et al., 2013) (H. Li, Lu, Chen, & Liu, 2017)
	MT-1E	ICC	downregulation	(Tarapore, et al., 2011)
	MT-1F	ICC	downregulation	(Tarapore, et al., 2011)
	MT-1G	ICC HCC HCC HCC Hepatocytes from primary HCC	downregulation downregulation, methylation downregulation, allelic lost downregulation upregulation	(Tarapore, et al., 2011) (Kanda, et al., 2009) (K. Y. Y. Chan, et al., 2006) (C. L. Fu, Pan, Pan, & Gan, 2017) (X. F. Sun, et al., 2016)
	MT-1H	Liver cancer ICC HCC	hypermethylation downregulation downregulation	(Han, et al., 2013) (Tarapore, et al., 2011) (Y. L. Zheng, et al., 2017)
	MT-1HL1	HCC	downregulation	(C. L. Fu, et al., 2017)
	MT-1IP	ICC	downregulation	(Tarapore, et

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				al., 2011)
	MT-1M	HCC serum from HCC patients	downregulation, hypermethylation downregulation hypermethylation	(J. Mao, et al., 2012) (C. L. Fu, et al., 2017) (Ji, et al., 2014)
	MT-1X	ICC	downregulation	(Tarapore, et al., 2011)
	MT-2A	HCC	downregulation	(X. Tao, Zheng, Xu, Chen, & Zhang, 2007)
Haematological malignancies	*MT-1E*	DLBCL ABC	upregulation	(Poulsen, et al., 2006)
	MT-1F	DLBCL ABC	upregulation	(Poulsen, et al., 2006)
	MT-1G	DLBCL ABC	upregulation	(Poulsen, et al., 2006)
	MT-1H	DLBCL ABC	upregulation	(Poulsen, et al., 2006)
	MT-1L	DLBCL ABC	upregulation	(Poulsen, et al., 2006)
	MT-1M	DLBCL ABC	upregulation	(Poulsen, et al., 2006)
	MT-1X	DLBCL ABC	upregulation	(Poulsen, et al., 2006)
	MT-2A	DLBCL ABC	upregulation	(Poulsen, et al., 2006)
	MT-3	AML	hypermethylation, downregulation	(Y. F. Tao, et al., 2014)
Head and neck cancer	*MT-1A*	OSCC	downregulation	(X. Yang, et al., 2014)
	MT-1E	OSCC	upregulation	(Brazao-Silva, et al., 2015)
	MT-1F	OSCC	upregulation	(Brazao-Silva, et al., 2015)
	MT-1G	ESCC OSCC	downregulation downregulation	(Kumar, et al., 2007) (Brazao-Silva, et al., 2015)
	MT-1H	OSCC	downregulation	(Brazao-Silva, et al., 2015)
	MT-1M	ESCC SCC	downregulation, hypermethylation hypermethylation	(Oka, et al., 2009) (Y. C. Lee, et al., 2011)
	MT-1X	OSCC	downregulation	(Brazao-Silva, et al., 2015)
	MT-2A	OSCC	upregulation	(Brazao-Silva, et al., 2015)
	MT-3	ESCC OSCC EAC	hypermethylation downregulation hypermethylation	(E. Smith, et al., 2005) (Brazao-Silva, et al., 2015) (D. F. Peng, et al., 2011)
	MT4	OSCC	downregulation	(Brazao-Silva, et al., 2015)
Endometrium cancer	*MT-1A*	p53 mutant UCEC	gene loss	(Delaney & Stupack, 2016)
	MT-1E	p53 mutant UCEC	gene loss	(Delaney & Stupack, 2016)
	MT-1F	p53 mutant UCEC	gene loss	(Delaney & Stupack, 2016)
	MT-1G	p53 mutant UCEC	gene loss	(Delaney &

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				Stupack, 2016)
	MT-1H	p53 mutant UCEC	gene loss	(Delaney & Stupack, 2016)
	MT-1X	p53 mutant UCEC	gene loss	(Delaney & Stupack, 2016)
	MT-3	p53 mutant UCEC	gene loss	(Delaney & Stupack, 2016)
Colorectal cancer	*MT-1A*	crc	downregulation	(Arriaga, et al., 2012)
	MT-1B	crc	downregulation	(Jansova, et al., 2006)
	MT-1E	crc	downregulation	(Arriaga, et al., 2012; Yan, et al., 2012)
	MT-1F	crc rectal adenocarcinoma after radiotherapy	downregulation upregulation	(Jansova, et al., 2006; Yan, et al., 2012) (Szelachowska, et al., 2012)
	MT-1G	crc	downregulation	(Arriaga, et al., 2012; Jansova, et al., 2006; Yan, et al., 2012)
	MT-1H	crc	downregulation	(Arriaga, et al., 2012; Jansova, et al., 2006; Yan, et al., 2012)
	MT-1M	crc	downregulation	(Arriaga, et al., 2012)
	MT-1X	crc crc rectal adenocarcinoma after radiotherapy	T20 repeat in unranslationed region downregulation upregulation	(Morandi, et al., 2012) (Arriaga, et al., 2012; Yan, et al., 2012) (Szelachowska, et al., 2012)
	MT-2A	crc rectal adenocarcinoma after radiotherapy	downregulation upregulation	(Jansova, et al., 2006) (Arriaga, et al., 2012) (Szelachowska, et al., 2012)
CNS tumours	*MT-1A*	short survival glioblastoma multiforme	upregulation	(Mehrian-Shai, et al., 2015)
	MT-1B	bone marrow from neuroblastoma patients short survival glioblastoma multiforme	overexpression upregulation	(Scaruffi, et al., 2012) (Mehrian-Shai, et al., 2015)
	MT-1E	bone marrow from neuroblastoma patients short survival glioblastoma multiforme	overexpression upregulation	(Scaruffi, et al., 2012) (Mehrian-Shai, et al., 2015)
	MT-1F	short survival glioblastoma multiforme	upregulation	(Mehrian-Shai, et al., 2015)
	MT-1G	bone marrow from neuroblastoma patients	overexpression	(Scaruffi, et al., 2012)
	MT-1H	bone marrow from neuroblastoma patients short survival glioblastoma multiforme	overexpression upregulation	(Scaruffi, et al., 2012) (Mehrian-Shai, et al., 2015)
	MT-1HL1	bone marrow from neuroblastoma patients	overexpression	(Scaruffi, et al., 2012)
	MT-1L	bone marrow from neuroblastoma patients short survival glioblastoma multiforme	overexpression upregulation	(Scaruffi, et al., 2012) (Mehrian-Shai,

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				et al., 2015)
	MT-1X	bone marrow from neuroblastoma patients	overexpression	(Scaruffi, et al., 2012)
	MT-2A	bone marrow from neuroblastoma patients	overexpression	(Scaruffi, et al., 2012)
	MT-3	short survival glioblastoma multiforme	upregulation	(Mehrian-Shai, et al., 2015)
Bladder cancer	*MT-1X*	bladder cancer	upregulation	(Somji, et al., 2001)

Abbreviations: CaP – prostate cancer, NSLC – non-small cell lung cancer, LLC – lung large-cell carcinoma, SCC – squamous cell carcinoma, BCC – basal cell carcinoma, RCC – renal cell carcinoma, APA - adrenocortical aldosterone-producing adenoma, ICC – intrahepatic cholangiocarcinoma, HCC – hepatocellular carcinoma, DLBCL – diffuse large B-cell lymphoma, ABC – activated B-cell, AML – acute myeloid leukaemia, OSCC – oral squamous cell carcinoma. ESCC – oesophageal squamous cell carcinoma, EAC – oesophageal adenocarcinoma, UCEC – uterine corpus endometrial carcinoma, CRC – colorectal cancer.

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Table 3. Summary of MTs (sub)isoforms expression studies in human prostate cancer cell lines. Up- and down regulation is related to non-treated cells, if not mentioned otherwise.

Gene	Cell line	Treatment	Observation	Citation
MT-1A	LNCaP	C/EBP alpha expression Zn^{2+} and Cd^{2+} Hypoxia	downregulation upregulation upregulation	(Yin, Smith, & Glass, 2005) (Hasumi, et al., 2003) (Yamasaki, Nomura, Sato, & Mimata, 2007)
	PC-3	C/EBP alpha expression Zn^{2+} and Cd^{2+} Hypoxia	downregulation upregulation upregulation	(Yin, et al., 2005) (Hasumi, et al., 2003) (Yamasaki, et al., 2007)
	RWPE-1	Cu^{2+} Cd^{2+}	upregulation upregulation	(Bigagli, Luceri, Bernardini, Dei, & Dolara, 2010) (Albrecht, et al., 2008)
MT-1B	LNCaP	C/EBP alpha expression	downregulation	(Yin, et al., 2005)
	PC-3	C/EBP alpha expression	downregulation	(Yin, et al., 2005)
	RWPE-1	Cu^{2+}	upregulation	(Bigagli, et al., 2010)
	VCAP	Disulfiram	downregulation	(Iljin, et al., 2009)
	LTL313h (XG)	Genistein	upregulation	(Nakamura, et al., 2013)
MT-1E	RWPE-1	Cu^{2+} Zn^{2+} or Cd^{2+} in presence of Ca^{2+}	upregulation Ca^{2+} -modified regulation	(Bigagli, et al., 2010) (Singh, et al., 2008)
	LTL313h (XG)	Genistein	upregulation	(Nakamura, et al., 2013)
	DU-145	MIC-1	downregulation	(T. Liu, et al., 2003)
MT-1F	LNCaP	C/EBP alpha expression	downregulation	(Yin, et al., 2005)
	PC-3	C/EBP alpha expression	downregulation	(Yin, et al., 2005)
	RWPE-1	Zn^{2+} and Cd^{2+}	upregulation	(Albrecht, et al., 2008)
	VCAP	Disulfiram	upregulation	(Iljin, et al., 2009)
MT-1G	LNCaP	Zn^{2+}	upregulation	(D. J. Smith, et al., 2006)
	RWPE-1	Cu^{2+}	upregulation	(Bigagli, et

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		Zn^{2+} and Cd^{2+}	upregulation	al., 2010) (Albrecht, et al., 2008)
	VCAP	Disulfiram	downregulation	(Iljin, et al., 2009)
	C4-2	Zn^{2+}	upregulation	(D. J. Smith, et al., 2006)
MT-1H	LNCaP	C/EBP alpha expression	downregulation	(Yin, et al., 2005)
	PC-3	C/EBP alpha expression no treatment	downregulation promoter hypermethylation	(Yin, et al., 2005) (Han, et al., 2013)
	RWPE-1	Cu^{2+} Zn^{2+} and Cd^{2+}	upregulation upregulation	(Bigagli, et al., 2010) (Albrecht, et al., 2008)
	LTL313h (XG)	Genistein	upregulation	(Nakamura, et al., 2013)
	DU-145	no treatment	promoter hypermethylation	(Han, et al., 2013)
MT-1JP	PC-3	Zn^{2+}	upregulation	(Lin, Wei, Maeder, Franklin, & Feng, 2009)
MT-1L	LNCaP C4-2	Zn^{2+}	upregulation	(D. J. Smith, et al., 2006)
MT-1M	PC-3	Zn^{2+}	upregulation	(Lin, et al., 2009)
	RWPE-1	Cu^{2+}	upregulation	(Bigagli, et al., 2010)
MT-1X	LNCaP	Zn^{2+} and Cd^{2+} Hypoxia	upregulation upregulation	(Hasumi, et al., 2003) (Yamasaki, et al., 2007)
	PC-3	Hypoxia	upregulation	(Yamasaki, et al., 2007)
	RWPE-1	Zn^{2+} or Cd^{2+} in presence of Ca^{2+}	Ca^{2+}-modified regulation	(Singh, et al., 2008)
	VCAP	Disulfiram	downregulation	(Iljin, et al., 2009)
	LTL313h (XG)	Genistein	upregulation	(Nakamura, et al., 2013)
	LAPC-4	Genistein 17β-Estradiol	upregulation downregulation	(Raschke, Rowland, Magee, & Pool-Zobel, 2006) (Raschke, et al., 2006)
MT-2A	LNCaP	C/EBP alpha expression Zn^{2+} and Cd^{2+} Zn^{2+} Hypoxia	downregulation upregulation upregulation upregulation	(Yin, et al., 2005) (Hasumi, et al., 2003) (D. J.

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				Smith, et al., 2006) (Yamasaki, et al., 2007)
	PC-3	C/EBP alpha expression Zn^{2+} and Cd^{2+} Hypoxia	downregulation upregulation upregulation	(Yin, et al., 2005) (Hasumi, et al., 2003) (Yamasaki, et al., 2007)
	RWPE-1	Zn^{2+} Zn^{2+} or Cd^{2+} in presence of Ca^{2+}	upregulation Ca^{2+} -modified regulation	(Bigagli, et al., 2010) (Singh, et al., 2008)
	VCAP	Disulfiram	downregulation	(Iljin, et al., 2009)
	LTL313h (XG)	Genistein	upregulation	(Nakamura, et al., 2013)
	C4-2	Zn^{2+}	upregulation	(D. J. Smith, et al., 2006)
	EPN	Raloxifene	upregulation	(Rossi, et al., 2011)
MT-3	LNCaP	C/EBP alpha expression Androgen (R1881)/As_2O_3/Cd^{2+}	downregulation upregulation	(Yin, et al., 2005) (Juang, et al., 2013)
	PC-3	C/EBP alpha expression Zn^{2+}	downregulation upregulation	(Yin, et al., 2005) (Lin, et al., 2009)

Abbreviations: XG – xenograft, MIC-1 – macrophage inhibitory cytokine 1, C/EBP alpha – CCAAT/enhancer-binding protein alpha, R1881 – methyltrienolone, synthetic androgen

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Table 4. Summary of MTs (sub)isoforms expression studies in human lung cancer cell lines. Up- and down regulation is related to non-treated cells, if not mentioned otherwise.

Gene	Cell line	Treatment	Observation	Citation
MT-1A	NCI-H526	Titanocene C	upregulation	(Olszewski, et al., 2011)
	SAE	THC	upregulation	(Sarafian, et al., 2005)
MT-1B	NCI-H526	Titanocene C	upregulation	(Olszewski, et al., 2011)
MT-1E	NCI-H526	Titanocene C	upregulation	(Olszewski, et al., 2011)
	LLC HOP92	no treatment	upregulation	(da Motta, et al., 2015)
MT-1F	NCI-H526	Titanocene C	upregulation	(Olszewski, et al., 2011)
	LLC HOP92	no treatment	upregulation	(da Motta, et al., 2015)
	A-549	MGd Acrolein	up-regulation downregulation	(Magda, et al., 2005) (Thompson & Burcham, 2008)
MT-1G	NCI-H526	Titanocene C	upregulation	(Olszewski, et al., 2011)
	LLC HOP92	no treatment	upregulation	(da Motta, et al., 2015)
	A-549	MGd cisPt resistance Rosiglitazone Carboplatin Rosiglitazone and carboplatin GW1892	up-regulation promoter hypermethylation downregulation upregulation downregulation downregulation	(Magda, et al., 2005) (Guo, et al., 2013) (Girnun, et al., 2007) (Girnun, et al., 2007) (Girnun, et al., 2007) (Girnun, et al., 2007)
MT-1H	NCI-H526	Titanocene C	upregulation	(Olszewski, et al., 2011)
	SAE	THC	upregulation	(Sarafian, et al., 2005)
	LLC HOP92	no treatment	upregulation	(da Motta, et al., 2015)

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	A-549	MGd cisPt resistance Acrolein Rosiglitazone Carboplatin Rosiglitazone and carboplatin GW1892	upregulation up-regulation downregulation downregulation upregulation downregulation downregulation	(Magda, et al., 2005) (Hou, Fan, Wang, & Lu, 2009) (Thompson & Burcham, 2008) (Girnun, et al., 2007) (Girnun, et al., 2007) (Girnun, et al., 2007) (Girnun, et al., 2007)
MT-1HL1	A-549	MGd	upregulation	(Magda, et al., 2005)
MT-1JP	NCI-H526	Titanocene C	upregulation	(Olszewski, et al., 2011)
	A-549	Acrolein	downregulation	(Thompson & Burcham, 2008)
MT-1L	A-549	MGd Acrolein Rosiglitazone Carboplatin Rosiglitazone and carboplatin GW1892	upregulation downregulation downregulation upregulation downregulation downregulation	(Magda, et al., 2005) (Thompson & Burcham, 2008) (Girnun, et al., 2007) (Girnun, et al., 2007) (Girnun, et al., 2007) (Girnun, et al., 2007)
MT-1M	LLC HOP92	no treatment	upregulation	(da Motta, et al., 2015)
MT-1X	NCI-H526	Titanocene C	upregulation	(Olszewski, et al., 2011)
	LLC HOP92	no treatment	upregulation	(da Motta, et al., 2015)
	A-549	MGd Acrolein Rosiglitazone Carboplatin Rosiglitazone and carboplatin GW1892	upregulation downregulation upregulation downregulation upregulation downregulation	(Magda, et al., 2005) (Thompson & Burcham, 2008) (Girnun, et al., 2007) (Girnun, et al., 2007) (Girnun, et al., 2007) (Girnun, et al., 2007)
MT-2A	NCI-H526	Titanocene C	upregulation	(Olszewski, et al., 2011)

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	SAE	THC	upregulation	(Sarafian, et al., 2005)
	LLC HOP92	no treatment	upregulation	(da Motta, et al., 2015)
	A549	MGd Acrolein Rosiglitazone Carboplatin Rosiglitazone and carboplatin GW1892	upregulation downregulation downregulation upregulation downregulation downregulation	(Magda, et al., 2005) (Thompson & Burcham, 2008) (Girnun, et al., 2007) (Girnun, et al., 2007) (Girnun, et al., 2007) (Girnun, et al., 2007)
	H-69 SW2	cisPt resistance	upregulation	(Y. Y. Yang, et al., 1994)
MT-3	A-549	Rosiglitazone Carboplatin Rosiglitazone and carboplatin	upregulation upregulation downregulation	(Girnun, et al., 2007) (Girnun, et al., 2007) (Girnun, et al., 2007)
	A-549 A-427 NCI-H358 H-292 H-23 H-522 H-1299 H322 H460	no treatment	downregulation due to GpG islands hypermethylation and histone acetylation	(Zhong, Fields, Su, Pan, & Robertson, 2007)

Abbreviations: SAE – small airway epithelial cells, THC – delta-9-tetrahydrocannabinol, MGd – motexafin gadolinium, GW1892 – PPAR gamma antagonist,

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Table 5. Summary of MTs (sub)isoforms expression studies in human breast cancer cell lines.

Up- and down regulation is related to non-treated cells, if not mentioned otherwise.

Gene	Cell line	Treatment	Observation	Citation
MT-1A	MCF-7	Ethanol	upregulation	(Gelfand, et al., 2017)
	MCF-10F	Parathion Estrogen Parathion and estrogen	downregulation no change downregulation	(Calaf & Roy, 2007) (Calaf & Roy, 2007) (Calaf & Roy, 2007)
	MCF-12A	Ethanol	upregulation	(Gelfand, Vernet, Bruhn, Vadgama, & Gonzalez-Cadavid, 2016)
	MDA-MB-231	no treatment Cd^{2+}	expression upregulation	(Tai, et al., 2003) (Sirchia, Longo, & Luparello, 2008)
	Hs 578T T-47D ZR-75-1	no treatment	expression	(Tai, et al., 2003)
MT-1B	MCF-7	Ethanol no treatment	upregulation no expression	(Gelfand, et al., 2017) (Tai, et al., 2003)
	MCF-12A	Ethanol	upregulation	(Gelfand, et al., 2016)
	MDA-MB-231	Cd^{2+} no treatment	no expression no expression	(Sirchia, et al., 2008) (Tai, et al., 2003)
	Hs 578T T-47D ZR-75-1	no treatment	no expression	(Tai, et al., 2003)
	C3.6	EGF HRG	upregulation upregulation	(Worthington, Bertani, Chan, Gerrits, & Timms, 2010) (Worthington, et al., 2010)
MT-1E	MCF-7	Cd^{2+} Melatonin Cd^{2+} and melatonin H_2O_2 TBH Menadione Zn^{2+} no treatment wtp53 silencing	upregulation downregulation upregulation downregulation downregulation upregulation upregulation no expression downregulation	(Alonso-Gonzalez, et al., 2008) (Alonso-Gonzalez, et al., 2008) (Alonso-Gonzalez, et al., 2008) (Chuang, et al., 2002) (Chuang, et al., 2002) (Chuang, et al., 2002) (Wierzowiecka, et al., 2016) (Friedline,

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				Garrett, Somji, Todd, & Sens, 1998; Tai, et al., 2003) (Ostrakhovitch, et al., 2016)
	MCF-10A	Cd^{2+}	upregulation	(Gurel, et al., 2005)
	MCF-10F	Parathion Estrogen Parathion and estrogen	downregulation no change downregulation	(Calaf & Roy, 2007) (Calaf & Roy, 2007) (Calaf & Roy, 2007)
	MDA-MB-231	Cd^{2+} Melatonin Cd^{2+} and melatonin Zn^{2+} no treatment	upregulation downregulation upregulation upregulation expression	(Alonso-Gonzalez, et al., 2008; Sirchia, et al., 2008) (Alonso-Gonzalez, et al., 2008) (Alonso-Gonzalez, et al., 2008) (Wierzowiecka, et al., 2016) (Friedline, et al., 1998; Tai, et al., 2003)
	Hs 578T	no treatment	expression	(Friedline, et al., 1998; Tai, et al., 2003)
	T-47D ZR-75-1	no treatment	no expression	(Friedline, et al., 1998; Tai, et al., 2003)
	HB2	Cd^{2+}	downregulation	(Sirchia & Luparello, 2009)
	PMC42	resistance to Cu^{2+} and Zn^{2+}	upregulation	(Barnes, Ackland, & Cornish, 2000)
	ME16C SK-BR-3	Zn^{2+}	upregulation	(Wierzowiecka, et al., 2016)
MT-1F	MCF-7	Cd^{2+} Melatonin Cd^{2+} and melatonin Ethanol PLU-1/JARID1B overexpression Zn^{2+} no treatment	downregulation upregulation downregulation upregulation downregulation upregulation expression	(Alonso-Gonzalez, et al., 2008) (Alonso-Gonzalez, et al., 2008) (Alonso-Gonzalez, et al., 2008) (Gelfand, et al., 2017) (Scibetta, et al., 2007) (Wierzowiecka, et al., 2016) (Tai, et al., 2003)

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	MCF-12A	Ethanol	upregulation	(Gelfand, et al., 2016)
	MDA-MB-231	Cd^{2+}	upregulation	(Alonso-Gonzalez, et al., 2008)
		Melatonin	downregulation	
		Cd^{2+} and melatonin	upregulation	
		Zn^{2+}	upregulation	(Alonso-Gonzalez, et al., 2008)
		no treatment	expression	
		Cd^{2+}	upregulation	(Alonso-Gonzalez, et al., 2008) (Wierzowiecka, et al., 2016) (Tai, et al., 2003) (Sirchia, et al., 2008)
	Hs 578T T-47D ZR-75-1	no treatment	expression	(Tai, et al., 2003)
	C3.6	EGF	upregulation	(Worthington, et al., 2010)
		HRG	upregulation	(Worthington, et al., 2010)
	ME16C SK-BR-3	Zn^{2+}	upregulation	(Wierzowiecka, et al., 2016)
MT-1G	MCF-7	Ethanol	upregulation	(Gelfand, et al., 2017)
		H_2O_2	upregulation	(Chuang, et al., 2002)
		TBH	downregulation	
		Menadione	upregulation	(Chuang, et al., 2002)
		Zn^{2+}	upregulation	(Wierzowiecka, et al., 2016)
		no treatment	no expression	(Tai, et al., 2003)
	MCF-10F	Parathion	downregulation	(Calaf & Roy, 2007)
		Estrogen	no change	(Calaf & Roy, 2007)
		Parathion and estrogen	downregulation	(Calaf & Roy, 2007)
	MCF-12A	Ethanol	upregulation	(Gelfand, et al., 2016)
	MDA-MB-231	Zn^{2+}	upregulation	(Wierzowiecka, et al., 2016)
		Cd^{2+}	expression	(Sirchia, et al., 2008)
		no treatment	no expression	(Tai, et al., 2003)
	MDA-MB-648	compared to BT-549 cell line	downregulation in MDA	(Tripathi, Misra, & Chaudhuri, 2005)
	Hs 578T T-47D ZR-75-1	no treatment	no expression	(Tai, et al., 2003)
	C3.6	EGF	upregulation	(Worthington, et al., 2010)
		HRG	upregulation	(Worthington, et al., 2010)
	ME16C	Zn^{2+}	upregulation	(Wierzowiecka, et al., 2016)
	SK-BR-3	Zn^{2+}	downregulation	(Wierzowiecka,

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				et al., 2016)
MT-1H	MCF-7	Ethanol	upregulation	(Gelfand, et al., 2017)
		H_2O_2	upregulation	(Chuang, et al., 2002)
		TBH	downregulation	(Chuang, et al., 2002)
		Menadione	upregulation	(Chuang, et al., 2002)
		PLU-1/JARID1B overexpression	downregulation	(Scibetta, et al., 2007)
		no treatment	expression	(Tai, et al., 2003)
	MCF-10F	Parathion	downregulation	(Calaf & Roy, 2007)
		Estrogen	no change	(Calaf & Roy, 2007)
		Parathion and estrogen	downregulation	(Calaf & Roy, 2007)
	MCF-12A	Ethanol	upregulation	(Gelfand, et al., 2016)
	MDA-MB-231	no treatment	expression	(Tai, et al., 2003)
		Cd^{2+}	no expression	(Sirchia, et al., 2008)
	Hs 578T T-47D ZR-75-1	no treatment	expression	(Tai, et al., 2003)
	C3.6	EGF	upregulation	(Worthington, et al., 2010)
		HRG	upregulation	(Worthington, et al., 2010)
MT-1L	MCF-7	Ethanol	upregulation	(Gelfand, et al., 2017)
		H_2O_2	upregulation	(Chuang, et al., 2002)
		TBH	downregulation	(Chuang, et al., 2002)
		Menadione	upregulation	(Chuang, et al., 2002)
	MCF-12A	Ethanol	upregulation	(Gelfand, et al., 2016)
	MDA-MB-648	compared to BT-549	downregulation in MDA	(Tripathi, et al., 2005)
	HB2	Cd^{2+}	downregulation	(Sirchia & Luparello, 2009)
MT-1M	C3.6	EGF	upregulation	(Worthington, et al., 2010)
		HRG	upregulation	(Worthington, et al., 2010)
MT-1X	MCF-7	Cd^{2+}	upregulation	(Alonso-Gonzalez, et al., 2008)
		Melatonin	downregulation	(Alonso-Gonzalez, et al., 2008)
		Cd^{2+} and melatonin	upregulation	(Alonso-Gonzalez, et al., 2008)
		Ethanol	upregulation	(Gelfand, et al., 2017)
		H_2O_2	upregulation	(Chuang, et al., 2002)
		TBH	downregulation	(Chuang, et al., 2002)
		Menadione	upregulation	
		PLU-1/JARID1B overexpression	downregulation	
		Zn^{2+}	upregulation	
		no treatment	expression	
		wtp53 silencing	downregulation	

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				(Chuang, et al., 2002) (Scibetta, et al., 2007) (Wierzowiecka, et al., 2016) (Friedline, et al., 1998) (Tai, et al., 2003) (Ostrakhovitch, et al., 2016)
	MCF-10A	Cd^{2+}	upregulation	(Gurel, et al., 2005)
	MCF-10F	Parathion	downregulation	(Calaf & Roy, 2007)
		Estrogen	upregulation	(Calaf & Roy, 2007)
		Parathion and estrogen	downregulation	(Calaf & Roy, 2007)
	MCF-12A	Ethanol	upregulation	(Gelfand, et al., 2017)
	MDA-MB-231	Cd^{2+}	upregulation	(Alonso-Gonzalez, et al., 2008)
		Melatonin	downregulation	(Alonso-Gonzalez, et al., 2008)
		Cd^{2+} and melatonin	upregulation	(Alonso-Gonzalez, et al., 2008)
		no treatment	expression	(Friedline, et al., 1998) (Tai, et al., 2003) (Wierzowiecka, et al., 2016)
		Zn^{2+}	upregulation	
	Hs 578T T-47D ZR-75-1	no treatment	expression	(Tai, et al., 2003) (Friedline, et al., 1998)
	PMC42	Cu^{2+} and Zn^{2+} resistance	upregulation	(Barnes, et al., 2000)
	ME16C SK-BR-3	Zn^{2+}	upregulation	(Wierzowiecka, et al., 2016)
	C3.6	EGF	upregulation	(Worthington, et al., 2010)
		HRG	upregulation	(Worthington, et al., 2010)
MT-2A	MCF-7	HIPK2 depletion	upregulation	(Puca, et al., 2009)
		Cd^{2+}	upregulation	(Alonso-Gonzalez, et al., 2008)
		Cd^{2+} and melatonin	downregulation	(Alonso-Gonzalez, et al., 2008)
		no treatment	upregulation	(Alonso-Gonzalez, et al., 2008)
		Ethanol	upregulation	(Gelfand, et al., 2017)
		H_2O_2	upregulation	(Chuang, et al., 2002)
		TBH	downregulation	(Chuang, et al., 2002)
		Menadione	upregulation	(Chuang, et al., 2002)
		Zn^{2+}	upregulation	
		no treatment	expression	
		wtp53 silencing	expression	
		wtp53 silencing and Cu^{2+} exposition	downregulation loss of expression sensitivity	
		MT-2A knock-out	proliferation and cell cycle arrest	

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				(Wierzowiecka, et al., 2016) (Wierzowiecka, et al., 2016) (Tai, et al., 2003) (Ostrakhovitch, et al., 2016) (Ostrakhovitch, et al., 2016) (Lim, Jocelyn, Yip, & Bay, 2009)
	MCF-10A	Cd^{2+}	upregulation	(Gurel, et al., 2005)
	MCF-10F	Parathion Estrogen Parathion and estrogen	downregulation no change downregulation	(Calaf & Roy, 2007) (Calaf & Roy, 2007) (Calaf & Roy, 2007)
	MCF-12A	Ethanol	upregulation	(Gelfand, et al., 2016)
	MDA-MD-231	Cd^{2+} Melatonin Cd^{2+} and melatonin MT-2A overexpression Zn^{2+} no treatment Cd^{2+}	upregulation downregulation upregulation invasivity, MMP-9 upregulation upregulation expression downregulation	(Alonso-Gonzalez, et al., 2008) (Alonso-Gonzalez, et al., 2008) (Alonso-Gonzalez, et al., 2008) (H. G. Kim, et al., 2011) (Wierzowiecka, et al., 2016) (Friedline, et al., 1998) (Tai, et al., 2003) (Sirchia, et al., 2008)
	Hs 578T T-47D ZR-75-1	no treatment	expression	(Friedline, et al., 1998; Tai, et al., 2003)
	PMC42	resistance to Cu^{2+} and Zn^{2+}	upregulation	(Barnes, et al., 2000)
	ME16C SK-BR-3	Zn^{2+}	upregulation	(Wierzowiecka, et al., 2016)
	HB2	Cd^{2+}	downregulation	(Sirchia & Luparello, 2009)
MT-3	MCF-7	Ethanol	upregulation	(Gelfand, et al., 2017)
	MDA-MB-231	Cd^{2+}	no expression	(Sirchia, et al., 2008)
	C3.6	EGF HRG	upregulation upregulation	(Worthington, et al., 2010) (Worthington, et al., 2010)
	HME	PEITC	upregulation	(Telang, Braeau, & Morris, 2009)
MT4	MCF-7	Ethanol	upregulation	(Gelfand, et al., 2017)
	MCF-12A	Ethanol	upregulation	(Gelfand, et al.,

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				2016)
	MDA-MB-231	Cd^{2+}	no expression	(Sirchia, et al., 2008)

Abbreviations: EGF – epithelial growth factor, HRG – heregulin, TBH - *t*-butyl hydroperoxide, PLU/JARID18 – transcriptional repressor, member of ARID DNA binding proteins, PEITC - Phenethyl isothiocyanate, HIPK2 - Homeodomain-interacting protein kinase 2,

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Table 6. Summary of MTs (sub)isoforms expression studies in human colorectal cancer cell lines. Up- and down regulation is related to non-treated cells, if not mentioned otherwise.

Gene	Cell line	Treatment	Observation	Citation
MT-1A	CaCo-2	Arsenic species	upregulation	(Calatayud, Devesa, & Velez, 2013)
MT-1B	CaCo-2	Gold nanoparticles Arsenic species	upregulation upregulation	(Bajak, et al., 2015) (Calatayud, et al., 2013)
	WiDr	SPINK1 knock-down	upregulation	(Tiwari, et al., 2015)
MT-1E	CaCo2	Rosiglitazone and/or AS601245 Gold nanoparticles	upregulation upregulation	(Cerbone, et al., 2012) (Bajak, et al., 2015)
	WiDr	SPINK1 knock-down	upregulation	(Tiwari, et al., 2015)
MT-1F	CaCo-2	Rosiglitazone and/or AS601245	upregulation	(Cerbone, et al., 2012)
	RKO	MT-1F transfection no treatment	inhibition of tumorigenicity hypermethylation	(Yan, et al., 2012)
	LoVo	no treatment	hypermethylation	(Yan, et al., 2012)
MT-1G	CaCo-2	Rosiglitazone and/or AS601245	upregulation	(Cerbone, et al., 2012)
	WiDr	SPINK1 knock-down	upregulation	(Tiwari, et al., 2015)
	HT-29	Tumour tissue DNA MT-1G transfection and Zn^{2+} MT-1G overexpression	upregulation chemotherapy sensitization tumour suppression differential genes regulation	(Furi, et al., 2015) (Arriaga, Greco, Mordoh, & Bianchini, 2014) (Arriaga, Bravo, Mordoh, & Bianchini, 2017)
	HCT-116	MT-1G transfection and Zn^{2+}	chemotherapy sensitization	(Arriaga, et al., 2014)
MT-1H	CaCo-2	15-lipoxygenase-1 expresion Rosiglitazone and/or AS601245 Taurine	upregulation upregulation upregulation	(Nixon, Kim, Lamb, Bottone, & Eling, 2004) (Cerbone, et al., 2012) (Gondo, Satsu, Ishimoto, Iwamoto, & Shimizu, 2012)
	WiDr	SPINK1 knock-down TPPS2a	upregulation upregulation	(Tiwari, et al., 2015) (Prasmickaite, et al., 2006)
	HT-29	Tumour tissue DNA	upregulation	(Furi, et al., 2015)
	MSI crc	no treatment	upregulation	(Giacomini, et al., 2005)

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MT-1HL1	CaCo-2	Rosiglitazone and/or AS601245	upregulation	(Cerbone, et al., 2012)
	HT-29	Tumour tissue DNA	downregulation	(Furi, et al., 2015)
MT-1L	CaCo-2	15-lipoxygenase-1 expresion	upregulation	(Nixon, et al., 2004)
	WiDr	SPINK1 knock-down	upregulation	(Tiwari, et al., 2015)
MT-1M	CaCo-2	Rosiglitazone and/or AS601245	upregulation	(Cerbone, et al., 2012)
	WiDr	SPINK1 knock-down	upregulation	(Tiwari, et al., 2015)
MT-1X	CaCo-2	Rosiglitazone and/or AS601245 Gold nanoparticles	upregulation upregulation	(Cerbone, et al., 2012) (Bajak, et al., 2015)
	WiDr	TPPS2a SPINK1 knock-down	upregulation upregulation	(Prasmickaite, et al., 2006) (Tiwari, et al., 2015)
	HT-29	Tumour tissue DNA	upregulation	(Furi, et al., 2015)
	HCT-116	Butyrate	upregulation	(H. T. Tan, et al., 2008)
	MSI crc	no treatment	upregulation	(Giacomini, et al., 2005)
MT-2A	CaCo-2	Rosiglitazone and/or AS601245 Gold nanoparticles Arsenic species 15-lipoxygenase-1 expression	upregulation upregulation upregulation upregulation	(Cerbone, et al., 2012) (Bajak, et al., 2015) (Calatayud, et al., 2013) (Nixon, et al., 2004)
	WiDr	SPINK1 knock-down in WiDr cell line	upregulation	(Tiwari, et al., 2015)
	HT-29	Tumour tissue DNA Tea polyphenols	upregulation downregulation	(Furi, et al., 2015) (H. Y. Jin, Tan, Liu, & Ding, 2010)
	SW-480	Tea polyphenols	upregulation	(H. Y. Jin, et al., 2010)
	LoVo HCT-116	Tea polyphenols	downregulation	(H. Y. Jin, et al., 2010)
	MSI crc	no treatment	upregulation	(Giacomini, et al., 2005)

Abbreviations: SPINK1 - Serine Protease Inhibitor Kazal-Type 1, AS601245 – JNK inhibitor , TPPS2a - disulfonated meso-tetraphenylporphin, photosensitizer,

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Table 7. Summary of MTs (sub)isoforms expression studies in human hepatic cancer cell lines. Up- and down regulation is related to non-treated cells, if not mentioned otherwise.

Gene	Cell line	Treatment	Observation	Citation
MT-1A	Hep G2	Mutant thyroid hormone receptor Cd^{2+} Genistin and its glycosides SPIONs	downregulation upregulation upregulation upregulation	(Brazao-Silva, et al., 2015) (Fabbri, Urani, Sacco, Procaccianti, & Gribaldo, 2012) (Chung, et al., 2006) (He, et al., 2016)
	Huh-7	HCV core proteins expression	upregulation	(K. Li, Prow, Lemon, & Beard, 2002)
	Bel-7402	Tanshinone IIA	upregulation	(Dai, et al., 2012)
MT-1B	Hep G2	Cd^{2+} SPIONs	upregulation upregulation	(Cartularo, et al., 2015; Fabbri, et al., 2012) (He, et al., 2016)
	Huh-7	HCV core proteins expression Sorafenib	upregulation upregulation	(K. Li, et al., 2002) (Houessinon, et al., 2016)
MT-1DP	Hep G2	Mutant thyroid hormone receptor Cd2+	downregulation upregulation	(Rosen, Chan, & Privalsky, 2011) (Cartularo, et al., 2015)
	Huh-7	MT-1DP overexpression MT-1DP knock-down	tumour suppression FoxA1 downregulation	(Yu, et al., 2014)
	Bel-7402	YAP or RunX2 overexpression MT-1DP overexpression MT-1DP knock-down	downregulation tumour suppression FoxA1 downregulation	(Yu, et al., 2014)
	SMMC-7721	MT-1DP overexpression MT-1DP knock-down	tumour suppression FoxA1 downregulation	(Yu, et al., 2014)
MT-1E	Hep G2	Mutant thyroid hormone receptor Cd^{2+} Genistin and its glycosides	downregulation upregulation upregulation	(Rosen, et al., 2011) (Fabbri, et al., 2012) (Chung, et al., 2006)
	Huh-7	HCV core proteins expression Sorafenib	upregulation upregulation	(K. Li, et al., 2002) (Houessinon, et al., 2016)
MT-1F	Hep G2	Cd^{2+} SPIONs	upregulation upregulation	(Cartularo, et al., 2015; Fabbri, et al., 2012) (He, et al., 2016)
	Huh-7	HCV core proteins expression	upregulation	(K. Li, et al., 2002)

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MT-1G	Hep G2	Mutant thyroid hormone receptor SM22 alpha-transfection Cd^{2+} Sorafenib	downregulation upregulation upregulation upregulation	(Rosen, et al., 2011) (T. R. Kim, et al., 2010) (Cartularo, et al., 2015; Fabbri, et al., 2012) (X. F. Sun, et al., 2016)
	Huh-7	HCV core proteins expression Sorafenib	upregulation upregulation	(K. Li, et al., 2002) (Houessinon, et al., 2016; X. F. Sun, et al., 2016)
	Hep 3B	Sorafenib no treatment	upregulation downregulation, allelic lost	(X. F. Sun, et al., 2016) (K. Y. Y. Chan, et al., 2006)
	HLE PLC/PRF/5 Huh2	no treatment	downregulation, methylation	(Kanda, et al., 2009)
	PLC/PRF/5 SNU-387 SNU-389 SNU-423 SNU-449 SNU-475	no treatment	downregulation, allelic lost	(K. Y. Y. Chan, et al., 2006)
MT-1H	Hep G2	Cd^{2+} MT-1H overexpression	upregulation decrease of viability and invasivity via regulating Wnt pathway	(Cartularo, et al., 2015; Fabbri, et al., 2012) (Y. L. Zheng, et al., 2017)
	Huh-7 Hep 3B	HCV core proteins expression Sorafenib MT-1H overexpression	upregulation upregulation decrease of viability and invasivity via regulating Wnt pathway	(K. Li, et al., 2002) (Houessinon, et al., 2016) (Y. L. Zheng, et al., 2017)
MT-1HL1	Hep G2	Cd^{2+} SPIONs	upregulation upregulation	(Cartularo, et al., 2015) (He, et al., 2016)
MT-1JP	Hep G2	Cd^{2+}	upregulation	(Fabbri, et al., 2012)
MT-1L	Hep G2	Mutant thyroid hormone receptor Cd^{2+}	downregulation upregulation	(Rosen, et al., 2011) (Fabbri, et al., 2012)
	Huh-7	Sorafenib	upregulation	(Houessinon, et al., 2016)
MT-1M	Hep G2	no treatment Cd^{2+} SPIONs MT-1M overexpression MT-1M knock-down	hypermethylation downregulation upregulation tumour growth inhibition stimulation of tumour growth	(J. Mao, et al., 2012) (Cartularo, et al., 2015; Fabbri, et al., 2012) (He, et al., 2016) (C. L. Fu, et al., 2017)
	Huh-7	Sorafenib MT-1M overexpression	hypermethylation tumour growth inhibition	(Houessinon, et al., 2016)

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		MT-1M knock-down	stimulation of tumour growth	(C. L. Fu, et al., 2017)
	Bel-7402 Bel-7404 QGY-7701 QGY-7703 SMMC-7721 Focus Hep3B HepG2 PLC SKHep-1 YY-8103	no treatment	downregulation, hypermethylation	(J. Mao, et al., 2012)
MT-1P3	Hep G2	Cd^{2+}	upregulation	(Cartularo, et al., 2015)
MT-1X	Hep G2	Cd^{2+} MT-1X knock-out Genistin and its glycosides SPIONs)	upregulation FHL3-dependent growth inhibition upregulation upregulation	(Cartularo, et al., 2015; Fabbri, et al., 2012) (Cai, et al., 2014) (Chung, et al., 2006) (He, et al., 2016)
MT-2A	Hep G2	Pb^{2+} Cd^{2+} Genistin and its glycosides SPIONs	upregulation upregulation upregulation upregulation	(Tchounwou, Yedjou, Foxx, Ishaque, & Shen, 2004) (Fabbri, et al., 2012) (Chung, et al., 2006) (He, et al., 2016)
	VL17A	Ethanol and/or Zn^{2+}	upregulation	(Liuzzi & Yoo, 2013)
MT-3	Huh-7	HCV core proteins expression	upregulation	(K. Li, et al., 2002)

Abbreviations: SPIONs – superparamagnetic iron oxide nanoparticles, HCV – hepatitis C virus, SMM22 alpha - Smooth muscle protein 22-alpha, Yap - Yes associated protein, RunX2 - Runt related transcription factor 2

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Table 8. Summary of MTs (sub)isoforms expression studies in human head and neck cancer cell lines. Up- and down regulation is related to non-treated cells, if not mentioned otherwise.

Gene	Cell line	Treatment	Observation	Citation
MT-1A	CNE-2 HK1 TW01 HEp-2	no treatment	no expression	(O. J. K. Tan, et al., 2005)
	OE33	HNF1A-AS1-knock-down	downregulated	(Rosen, et al., 2011)
MT-1B	CNE-2 HK1 TW01 HEp-2	no treatment	no expression	(O. J. K. Tan, et al., 2005)
	Tca8113	Pingyangmycin resistance	upregulation	(G. P. Zheng, et al., 2010)
MT-1E	CNE-2	no treatment	no expression	(O. J. K. Tan, et al., 2005)
	HK1 TW01 HEp-2	no treatment	expression	(O. J. K. Tan, et al., 2005)
	OE33	HNF1A-AS1-knock-down	downregulated	(X. Yang, et al., 2014)
	HK1 NPC	Hypericin	upregulation	(Du, Li, Olivo, Yip, & Bay, 2006)
	SCC25	cisPt resistance	upregulation	(Y. Y. Yang, et al., 1994)
	Eca-109 TE-13	MT-1E-transfection	no apoptosis/ proliferation effect	(Tian, et al., 2013)
MT-1F	CNE-2 HK1 TW01 HEp-2	no treatment	no expression	(O. J. K. Tan, et al., 2005)
	HepG2	Mutant thyroid receptor	downregulated	(Rosen, et al., 2011)
MT-1G	CNE-2 HK1 TW01 HEp-2	no treatment	no expression	(O. J. K. Tan, et al., 2005)
	HepG2	Mutant thyroid receptor	downregulated	(Rosen, et al., 2011)
	Tca8113	Pingyangmycin resistance	upregulation	(G. P. Zheng,

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				et al., 2010)
MT-1H	CNE-2 HK1 TW01 HEp-2	no treatment	no expression	(O. J. K. Tan, et al., 2005) (O. J. K. Tan, et al., 2005) (O. J. K. Tan, et al., 2005) (O. J. K. Tan, et al., 2005)
MT-1M	KYSE30 KYSE220 KYSE270	no treatment	downregulated, methylated	(Oka, et al., 2009)
MT-1X	CNE-2 HK1 TW01 HEp-2	no treatment	no expression	(O. J. K. Tan, et al., 2005)
	Tca8113	TCRP-1 knock-down Pingyangmycin resistance	downregulation	(B. Peng, Gu, Xiong, Zheng, & He, 2012)
MT-2A	CNE-2 HK1 TW01 HEp-2	no treatment	expression	(O. J. K. Tan, et al., 2005)
	OE33	HNF1A-AS1-knock-down	downregulated	(X. Yang, et al., 2014)
	Tca8113	Pingyangmycin resistance	upregulation	(G. P. Zheng, et al., 2010)
	HK1 NPC	Hypericin	upregulation	(Du, et al., 2006)
	SCC-25	cisPt resistance	upregulation	(Y. Y. Yang, et al., 1994)
MT-3	CNE-2 HK1 TW01 HEp-2	no treatment	no expression	(O. J. K. Tan, et al., 2005)
	OE19 OE21 OE33 TE-7	no treatment	promoter methylation, no expression	(E. Smith, et al., 2005)
	OE19 OE21 TE-7	no treatment	downregulation	(E. Smith, et al., 2005)

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	SCC-25	EGCG	no change in regulation	(L. Tao, Forester, & Lambert, 2014)
	NGF-1	(EGCG	upregulation	(L. Tao, et al., 2014)
	Eca-109 TE-13	MT-3-transfection	inhibited proliferation, apoptosis	(Tian, et al., 2013)
MT4	CNE-2 HK1 TW01 HEp-2	no treatment	no expression	(O. J. K. Tan, et al., 2005)

Abbreviations: HNF1A-AS1 - HNF1A antisense RNA 1, TCRP-1 - tongue cancer resistance-associated protein 1, EGCG - (-)-epigallocatechin-3-gallate, green tea catechin

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Table 9. Summary of MTs (sub)isoforms expression studies in human haematological cancer cell lines. Up- and down regulation is related to non-treated cells, if not mentioned otherwise.

Gene	Cell line	Treatment	Observation	Citation
MT-1A	K-562 DAMI MEG-01 ELF-153	Zn^{2+}	upregulation	(Bagheri, Rahman, Van Soest, & De Ley, 2009)
	K-562	PMA	downregulation	(Bagheri, et al., 2009)
	NB4	Nucleostemin knock-out	downregulation	(Sun, Jia, Wei, Liu, & Yue, 2016)
	DoHH-2 TMD8	ITF-A	upregulation	(Mensah, et al., 2015)
MT-1B	K-562 DAMI MEG-01	Zn^{2+}	upregulation	(Bagheri, et al., 2009)
	K-562	PMA	downregulation	(Bagheri, et al., 2009)
	NB4	Nucleostemin knock-out	downregulation	(X. L. Sun, et al., 2016)
MT-1E	K-562 DAMI MEG-01	Zn^{2+}	upregulation	(Bagheri, et al., 2009)
	K-562	PMA	upregulation	(Bagheri, et al., 2009)
	NB4	Nucleostemin knock-out	downregulation	(X. L. Sun, et al., 2016)
	DoHH-2 TMD8	ITF-A	upregulation	(Mensah, et al., 2015)
MT-1F	K-562 DAMI MEG-01 ELF-153	Zn^{2+}	upregulation	(Bagheri, et al., 2009)
	K-562	PMA	downregulation	(Bagheri, et al., 2009)
	NB4	Nucleostemin knock-out	downregulation	(X. L. Sun, et al., 2016)
	DoHH-2 TMD8	ITF-A	upregulation	(Mensah, et al., 2015)
MT-1G	K-562 DAMI MEG-01 ELF-153	Zn^{2+}	upregulation	(Bagheri, et al., 2009)
	K-562	PMA	downregulation	(Bagheri, et al., 2009)
	DoHH-2 TMD8	ITF-A	upregulation	(Mensah, et al., 2015)
MT-1H	K-562 DAMI MEG-01	Zn^{2+}	upregulation	(Bagheri, et al., 2009)
	K-562	PMA	downregulation	(Bagheri, et

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				al., 2009)
	NB4	Nucleostemin knock-out	downregulation	(X. L. Sun, et al., 2016)
	DoHH-2 TMD8	ITF-A	upregulation	(Mensah, et al., 2015)
MT-1L	NB4	Nucleostemin knock-out	downregulation	(X. L. Sun, et al., 2016)
MT-1X	K-562 DAMI MEG-01 ELF-153	Zn^{2+}	upregulation	(Bagheri, et al., 2009)
	K-562	PMA	upregulation	(Bagheri, et al., 2009)
	NB4	Nucleostemin knock-out	downregulation	(X. L. Sun, et al., 2016)
	DoHH-2 TMD8	ITF-A	upregulation	(Mensah, et al., 2015)
MT-2A	K-562 DAMI MEG-01 ELF-153	Zn^{2+}	upregulation	(Bagheri, et al., 2009)
	K-562	PMA	upregulation	(Bagheri, et al., 2009)
	NB4	Nucleostemin knock-out	downregulation	(X. L. Sun, et al., 2016)
	DoHH-2 TMD8	ITF-A	upregulation	(Mensah, et al., 2015)
MT-3	HL-60 MV4-11 697 SHI1 K-562 U-937 THP-1 Raji NB-4 Jurkat Daudi	no treatment	methylation, downregulation	(Y. F. Tao, et al., 2014)

Abbreviations: PMA - phorbol-12 myristate-13 acetate, ITF-A – histone deacetylase inhibitor,

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Table 10. Summary of MTs (sub)isoforms expression studies in other human cancer cell lines. Up- and down regulation is related to non-treated cells, if not mentioned otherwise.

Diagnosis	Gene	Cell line	Treatment	Observation	Citation
CNS cancer	*MT-1A*	U-87	As_2O_3 for 48 h As_2O_3 for 48 h after 48 h recovery	downregulation upregulation	(Falnoga, et al., 2012)
		U-251	miR340-transfection miR1293-transfection	upregulation downregulation	(Cosset, et al., 2016)
		D-341	BCNU-resistance	upregulation	(Bacolod, et al., 2002)
	MT-1E	U-87	As_2O_3 for 48 h As_2O_3 for 48 h after 48 h recovery MT-1E knock-down	downregulation upregulation decreased motility and invasivity	(Falnoga, et al., 2012) (Falnoga, et al., 2012) (Ryu, et al., 2012)
		U-251	miR340-transfection miR1293-transfection	upregulation downregulation	(Cosset, et al., 2016)
		D-341	BCNU-resistance	upregulation	(Bacolod, et al., 2002)
		U-343	MT-1E knock-in	increased motility and invasivity	(Ryu, et al., 2012)
	MT-1F	U-87	As_2O_3 for 48 h As_2O_3 for 48 h after 48 h recovery	upregulation upregulation	(Falnoga, et al., 2012)
		U-251	miR340-transfection	upregulation	(Cosset, et al., 2016)
		D-341	BCNU-resistance	upregulation	(Bacolod, et al., 2002)
	MT-1H	U-251	miR340-transfection	upregulation	(Cosset, et al., 2016)
		SKNBE(2)	Hypoxia	upregulation	(Jogi, et al., 2004)
	MT-1L	D-341	BCNU-resistance	upregulation	(Bacolod, et al., 2002)
	MT-1X	U-87	As_2O_3 for 48 h As_2O_3 for 48 h after 48 h recovery	upregulation upregulation	(Falnoga, et al., 2012)
		U-251	miR340-transfection miR1293-transfection	upregulation downregulation	(Cosset, et al., 2016)
	MT-2A	U-87	As_2O_3 for 48 h As_2O_3 for 48 h after 48 h recovery	upregulation upregulation	(Falnoga, et al., 2012)
		U-251	miR340-transfection miR1293-transfection	upregulation downregulation	(Cosset, et al., 2016)
		D-341	BCNU-resistance	upregulation	(Bacolod, et al.,

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					2002)
		SKNBE(2)	Hypoxia	upregulation	(Jogi, et al., 2004)
	MT-3	U-87 SKNSH	As_2O_3 for 48 h As_2O_3 for 48 h after 48 h recovery MT-3 overexpression, γ-irradiation	upregulation no change 8-oxoG suppression	(Falnoga, et al., 2012) (Jeong, et al., 2004)
Thyroid cancer	*MT-1A*	KAT-5	Cd^{2+} Ca^{2+} or ERK1/2 inhibitor	upregulation downregulation	(Z. M. Liu, et al., 2009)
	MT-1B	KAT-5	Cd^{2+} Ca^{2+} or ERK1/2 inhibitor	upregulation downregulation	(Z. M. Liu, et al., 2009)
	MT-1E	KAT-5	Cd^{2+} Ca^{2+} or ERK1/2 inhibitor	upregulation downregulation	(Z. M. Liu, et al., 2009)
	MT-1F	KAT-5	Cd^{2+} Ca^{2+} or ERK1/2 inhibitor	upregulation downregulation	(Z. M. Liu, et al., 2009)
	MT-1G	KAT-5	Cd^{2+} Ca^{2+} or ERK1/2 inhibitor	upregulation downregulation	(Z. M. Liu, et al., 2009)
		NPA-87 K1 K2	no treatment	methylation	(Huang, et al., 2003)
		BCPAP FTC-133 IHH4 K1 8305C C643	MT-1G transfection	hypermethylation tumour suppression via downregulation	(J. Fu, et al., 2013)
		K1	MT-1G transfection	increased growth and tumorigenicity	(Ferrario, et al., 2008)
	MT-1H	KAT-5	Cd^{2+} Ca^{2+} or ERK1/2 inhibitor	upregulation downregulation	(Z. M. Liu, et al., 2009)
	MT-1X	KAT-5	Cd^{2+} Ca^{2+} or ERK1/2 inhibitor	upregulation downregulation	(Z. M. Liu, et al., 2009)
		FTC-133	wtTSHR expressinon, TSH stimulation	upregulation	(Back, et al., 2013)
	MT-2A	KAT-5	Cd^{2+} Ca^{2+} or ERK1/2 inhibitor	upregulation downregulation	(Z. M. Liu, et al., 2009)
Renal cancer	*MT-1E*	HEK-293	As^{3+}	upregulation	(X. H. Zheng, Watts, Vaught, & Gandolf

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					i, 2003)
		A-498	DNA methylation inhibitor	upregulation	(Alkama l, et al., 2015)
	MT-1G	HEK-293	As^{3+}	upregulation	(X. H. Zheng, et al., 2003)
		A-498	DNA methylation inhibitor	upregulation	(Alkama l, et al., 2015)
	MT-1H	HEK-293	As^{3+}	upregulation	(X. H. Zheng, et al., 2003)
		A-498	DNA methylation inhibitor	upregulation	(Alkama l, et al., 2015)
	MT-1L	HEK-293	As^{3+}	upregulation	(X. H. Zheng, et al., 2003)
	MT-1M	A-498	DNA methylation inhibitor	upregulation	(Alkama l, et al., 2015)
	MT-1X	A-498	DNA methylation inhibitor	upregulation	(Alkama l, et al., 2015)
	MT-2A	HEK-293	As^{3+}	upregulation	(X. H. Zheng, et al., 2003)
		A-498	DNA methylation inhibitor	upregulation	(Alkama l, et al., 2015)
	MT-3	H295R	angiotensin II and forskolin	upregulation	(Felizol a, et al., 2014)
Stomach cancer	*MT-1F*	MKN-28	no treatment	expression	(Soo, et al., 2011)
	MT-1X	MKN-28	no treatment	expression	(Soo, et al., 2011)
	MT-2A	MKN-28	no treatment	expression	(Soo, et al., 2011)
		BGC-823 SGC-7901 MGC-803 AGS SNU-1 RF-1 RF-48	no treatment	downregulation	(Pan, Xing, et al., 2013)
		BGC-823 SGC-7901 AGS MT-2A-BGC-823	DATS and/or DOC	upregulation	(Pan, et al., 2016)
		SNU-1, -16, -216,-484, -601, -638, -668, -719	no treatment	downregulation	(J. M. Kim, et al., 2005)
		BGC-823 MGC-803 AGS	miR-23a transfection	downregulation	(An, et al., 2013)

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		GES-1			
	MT-3	AGS MKN-45	no treatment	hypermethylation	(Deng, et al., 2003)
Bladder cancer	*MT-1A*	5637	DBC1 expression	upregulation	(Louhelainen, et al., 2006)
		HTB-1 HTB-2 HTB-5 CRL-1472	no treatment	expression	(Garrett, Somji, et al., 1999)
	MT-1B	5637	DBC1 expression	upregulation	(Louhelainen, et al., 2006)
	MT-1E	SLT4	MT-1E overexpression	increased migration	(Wu, et al., 2008)
		HTB-5	no treatment	expression	(Garrett, Somji, et al., 1999)
	MT-1F *MT-1L* *MT-1M*	5637 5637 5637	DBC1 expression DBC1 expression DBC1 expression	upregulation upregulation upregulation	(Louhelainen, et al., 2006)
	MT-1X	HTB-1 HTB-2 HTB-5 CRL-1472	no treatment	expression	(Garrett, Somji, et al., 1999)
	MT-3	5637	DBC1 expression	upregulation	(Louhelainen, et al., 2006)
		HTB-1 HTB-2 HTB-5 CRL-1472	no treatment	expression	(Garrett, Somji, et al., 1999)
	MT4	CRL-1472	no treatment	expression	(Garrett, Somji, et al., 1999)
Cervical cancer	*MT-1A*	HeLa	Zn^{2+}, Cd^{2+}, As^{3+}	upregulation	(Miura & Koizumi, 2007)
	MT-1B	HeLa	Zn^{2+}, Cd^{2+}, As^{3+}	upregulation	(Miura & Koizumi, 2007)
	MT-1E	HeLa	Cd^{2+} Melatonin Cd^{2+} and melatonin Zn^{2+}, Cd^{2+}, As^{3+}	upregulation downregulation upregulation upregulation	(Alonso-Gonzalez, et al., 2008) (Miura & Koizumi, 2007)

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	MT-1F	HeLa	Cd^{2+} Melatonin Cd^{2+} and melatonin Zn^{2+}, Cd^{2+}, As^{3+}	upregulation downregulation upregulation upregulation	(Alonso-Gonzalez, et al., 2008) (Miura & Koizumi, 2007)
		Ecto1/E6E7	NKK	upregulation	(Prokopczyk, Sinha, Trushin, Freeman, & El-Bayoumy, 2009)
	MT-1G	HeLa	Zn^{2+}, Cd^{2+}, As^{3+}	upregulation	(Miura & Koizumi, 2007)
	MT-1H	HeLa	Zn^{2+}, Cd^{2+}, As^{3+}	upregulation	(Miura & Koizumi, 2007)
	MT-1X	HeLa	Cd^{2+} Melatonin Cd^{2+} and melatonin Zn^{2+}, Cd^{2+}, As^{3+}	upregulation downregulation upregulation upregulation	(Alonso-Gonzalez, et al., 2008) (Miura & Koizumi, 2007)
	MT-2A	HeLa	Cd^{2+} Melatonin Cd^{2+} and melatonin zinc-pyrithione Zn^{2+}, Cd^{2+}, As^{3+}	upregulation downregulation upregulation upregulation upregulation	(Alonso-Gonzalez, et al., 2008) (Rudolf & Cervinka, 2010) (Miura & Koizumi, 2007)
		Hep2	MT-2A knock-out, zinc-pyrithione	lysosomal disruption, apoptosis	(Rudolf & Cervinka, 2010)
	MT-3	HeLa	Zn^{2+}, Cd^{2+}, As^{3+}	upregulation	(Miura & Koizumi, 2007)
	MT4	HeLa	Zn^{2+}, Cd^{2+}, As^{3+}	upregulation	(Miura & Koizumi, 2007)
Testicular cancer	*MT-1H*	NT2/D1	STK17A knock-down	upregulation	(P. Mao, et al.,

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					2011)
	MT-1M	NT2/D1	STK17A knock-down	upregulation	(P. Mao, et al., 2011)
	MT-1X	NT2/D1	STK17A knock-down	upregulation	(P. Mao, et al., 2011)
Endometrial cancer	*MT-1A*	Ishikawa	Progesterone	upregulation	(Paulssen, Moe, Gronaas, & Orbo, 2008)
	MT-1B	Ishikawa	Progesterone RU486	upregulation upregulation	(Paulssen, et al., 2008) (Orbo, Moe, Gronaas, & Paulssen, 2009)
	MT-1E	Ishikawa	RU486	upregulation	(Orbo, et al., 2009)
		Non-specified	no treatment 5-azacytidine	downregulation restoring the normal regulation	(Tse, et al., 2009)
	MT-1F *MT-1G* *MT-1H*	Ishikawa Ishikawa Ishikawa	Progesterone Progesterone Progesterone	upregulation upregulation upregulation	(Paulssen, et al., 2008)
	MT-1L	Ishikawa	Progesterone Progesterone, PRA/B expression	upregulation upregulation	(Paulssen, et al., 2008) (Smid-Koopman, et al., 2005)
	MT-2A	Ishikawa	Progesterone	upregulation	(Paulssen, et al., 2008)
Ovarian cancer	*MT-2A*	2008 A2780 HEY IGROV1 KF UCI	cisPt resistance	upregulation upregulation downregulation upregulation upregulation upregulation	(Cheng, et al., 2006)
		SKOV3 OVCA432 OVCA433	MT-2A knock-down	proliferation inhibition	(Tarapore, et al., 2011)
Sarcona	*MT-2A*	SaOS2 SaOS2 U0OS	Atorvastatin MT-2A transfection	upregulation decreased viability (Zn chelation) increased cytostatics resistance	(Habel, et al., 2013)
		SaOS2 U0OS	MT-2A silencing	decreased differentiation	(Habel, et al., 2013)
Melanoma and non-melanoma skin cancers	*MT-1E*	WM-793	No treatment	gene methylation	(Faller, et al., 2010)
	MT-1G	1205Lu	irradiation	upregulation	(Sokolov, Panyutin, Panyutin, &

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				Neuman n, 2011)
MT-1H *MT-1L* *MT-1M*	hESCs H9	irradiation	upregulation	(Sokolo v, et al., 2011)
MT-2A	A2058	CT16 knock-down	upregulation	(Nylund , et al., 2012)

Abbreviations: BCNU - 1,3-bis(2-chloroethyl)-1-nitrosourea, ERK1/2 - extracellular signal-regulated kinase 1, TSHR - thyroid stimulating hormone receptor, TSH - thyroid stimulating hormone, DATS – diallyl trisulphide, DOC – docetaxel, DBC1 - deleted in bladder cancer protein 1, NKK - 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, tobacco carcinogen, STK17A - Serine/Threonine Kinase 17a, RU486 – mifepristone, PRA/B – Progesterone receptor isoform A, CT16 - cancer-testis antigen 16, 8-oxoG – 8-oxoguanine

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References

AbdelMageed, A. B., & Agrawal, K. C. (1997). Antisense down-regulation of metallothionein induces growth arrest and apoptosis in human breast carcinoma cells. *Cancer Gene Ther., 4*, 199-207.

Adams, S. V., Barrick, B., Christopher, E. P., Shafer, M. M., Makar, K. W., Song, X. L., Lampe, J. W., Vilchis, H., Ulery, A., & Newcomb, P. A. (2015). Genetic variation in metallothionein and metal-regulatory transcription factor 1 in relation to urinary cadmium, copper, and zinc. *Toxicol. Appl. Pharmacol., 289*, 381-388.

Albrecht, A. L., Singh, R. K., Somji, S., Sens, M. A., Sens, D. A., & Garrett, S. H. (2008). Basal and metal-induced expression of metallothionein isoform 1 and 2 genes in the RWPE-1 human prostate epithelial cell line. *J. Appl. Toxicol., 28*, 283-293.

Alkamal, I., Ikromov, O., Tolle, A., Fuller, T. F., Magheli, A., Miller, K., Krause, H., & Kempkensteffen, C. (2015). An Epigenetic Screen Unmasks Metallothioneins as Putative Contributors to Renal Cell Carcinogenesis. *Urol. Int., 94*, 99-110.

Alonso-Gonzalez, C., Mediavilla, D., Martinez-Campa, C., Gonzalez, A., Cos, S., & Sanchez-Barcelo, E. J. (2008). Melatonin modulates the cadmium-induced expression of MT-2 and MT-1 metallothioneins in three lines of human tumor cells (MCF-7, MDA-MB-231 and HeLa). *Toxicol. Lett., 181*, 190-195.

Alvarez, L., Gonzalez-Iglesias, H., Garcia, M., Ghosh, S., Sanz-Medel, A., & Coca-Prados, M. (2012). The Stoichiometric Transition from Zn6Cu1-Metallothionein to Zn-7-Metallothionein Underlies the Up-regulation of Metallothionein (MT) Expression: quantitative analysis of MT-metal load in eye cells. *J. Biol. Chem., 287*, 28456-28469.

An, J., Pan, Y. M., Yan, Z., Li, W. M., Cui, J. T., Yuan, J., Tian, L. Q., Xing, R., & Lu, Y. Y. (2013). MiR-23a in Amplified 19p13.13 Loci Targets Metallothionein 2A and Promotes Growth in Gastric Cancer Cells. *J. Cell. Biochem., 114*, 2160-2169.

Arentz, G., Mittal, P., Zhang, C., Ho, Y. Y., Briggs, M., Winderbaum, L., Hoffmann, M. K., & Hoffmann, P. (2017). Chapter Two - Applications of Mass Spectrometry Imaging to Cancer. In R. R. Drake & L. A. McDonnell (Eds.), *Advances in Cancer Research* (Vol. Volume 134, pp. 27-66): Academic Press.

Arriaga, J. M., Bravo, A. I., Mordoh, J., & Bianchini, M. (2017). Metallothionein 1G promotes the differentiation of HT-29 human colorectal cancer cells. *Oncol. Rep., 37*, 2633-2651.

Arriaga, J. M., Greco, A., Mordoh, J., & Bianchini, M. (2014). Metallothionein 1G and Zinc Sensitize Human Colorectal Cancer Cells to Chemotherapy. *Mol. Cancer Ther., 13*, 1369-1381.

Arriaga, J. M., Levy, E. M., Bravo, A. I., Bayo, S. M., Amat, M., Aris, M., Hannois, A., Bruno, L., Roberti, M. P., Loria, F. S., Pairola, A., Huertas, E., Mordoh, J., & Bianchini, M. (2012). Metallothionein expression in colorectal cancer: relevance of different isoforms for tumor progression and patient survival. *Hum. Pathol., 43*, 197-208.

Back, C. M., Stohr, S., Schafer, E. A. M., Biebermann, H., Boekhoff, I., Breit, A., Gudermann, T., & Buch, T. R. H. (2013). TSH induces metallothionein 1 in thyrocytes via G(q/11)- and PKC-dependent signaling. *J. Mol. Endocrinol., 51*, 79-90.

Bacolod, M. D., Johnson, S. P., Ali-Osman, F., Modrich, P., Bullock, N. S., Colvin, O. M., Bigner, D. D., & Friedman, H. S. (2002). Mechanisms of resistance to 1,3-bis(2-chloroethyl)-1-nitrosourea in human medulloblastoma and rhabdomyosarcoma. *Mol. Cancer Ther., 1*, 727-736.

ACCEPTED MANUSCRIPT

Bagheri, P. M., Rahman, M. T., Van Soest, S., & De Ley, M. (2009). Differential quantitative zinc-induced expression of human metallothionein isogenes in haematopoietic precursor cell lines. *J. Trace Elem. Med. Biol., 23*, 124-131.

Bajak, E., Fabbri, M., Ponti, J., Gioria, S., Ojea-Jimenez, I., Collotta, A., Mariani, V., Gilliland, D., Rossi, F., & Gribaldo, L. (2015). Changes in Caco-2 cells transcriptome profiles upon exposure to gold nanoparticles. *Toxicol. Lett., 233*, 187-199.

Barnes, N. L., Ackland, M. L., & Cornish, E. J. (2000). Metallothionein isoform expression by breast cancer cells. *Int J. Biochem. Cell Biol., 32*, 895-903.

Bienengraber, M., Forderkunz, S., Klein, D., & Summer, K. H. (1995). Determination of Cu-containing metallothionein: comparison of Ag saturation assay, thiomolybdate assay, and enzyme-linked immunosorbent assay. *Anal. Biochem., 228*, 69-73.

Bigagli, E., Luceri, C., Bernardini, S., Dei, A., & Dolara, P. (2010). Extremely low copper concentrations affect gene expression profiles of human prostate epithelial cell lines. *Chem.-Biol. Interact., 188*, 214-219.

Bogumil, R., Faller, P., Binz, P. A., Vasak, M., Charnock, J. M., & Garner, C. D. (1998). Structural characterization of Cu(I) and Zn(II) sites in neuronal-growth-inhibitory factor by extended X-ray absorption fine structure (EXAFS). *Eur. J. Biochem., 255*, 172-177.

Brazao-Silva, M. T., Rodrigues, M. F. S., Eisenberg, A. L. A., Dias, F. L., de Castro, L. M., Nunes, F. D., Faria, P. R., Cardoso, S. V., Loyola, A. M., & de Sousa, S. (2015). Metallothionein gene expression is altered in oral cancer and may predict metastasis and patient outcomes. *Histopathology, 67*, 358-367.

Bredel, M. (2001). Anticancer drug resistance in primary human brain tumors. *Brain Research Reviews, 35*, 161-204.

Cai, X., Wang, J. F., Huang, X., Fu, W. L., Xia, W. R., Zou, M. J., Wang, Y. Y., Wang, J. X., & Xu, D. G. (2014). Identification and Characterization of MT-1X as a Novel FHL3-Binding Partner. *Plos One, 9*, 1-8.

Calaf, G. M., & Roy, D. (2007). Human drug metabolism genes in parathion and estrogen-treated breast cells. *Int. J. Mol. Med., 20*, 875-881.

Calatayud, M., Devesa, V., & Velez, D. (2013). Differential toxicity and gene expression in Caco-2 cells exposed to arsenic species. *Toxicol. Lett., 218*, 70-80.

Cartularo, L., Laulicht, F., Sun, H., Kluz, T., Freedman, J. H., & Costa, M. (2015). Gene expression and pathway analysis of human hepatocellular carcinoma cells treated with cadmium. *Toxicol. Appl. Pharmacol., 288*, 399-408.

Cerbone, A., Toaldo, C., Minelli, R., Ciamporcero, E., Pizzimenti, S., Pettazzoni, P., Roma, G., Dianzani, M. U., Ullio, C., Ferretti, C., Dianzani, C., & Barrera, G. (2012). Rosiglitazone and AS601245 Decrease Cell Adhesion and Migration through Modulation of Specific Gene Expression in Human Colon Cancer Cells. *Plos One, 7*, 1-14.

Cosset, E., Petty, T., Dutoit, V., Tirefort, D., Otten-Hernandez, P., Farinelli, L., Dietrich, P. Y., & Preynat-Seauve, O. (2016). Human tissue engineering allows the identification of active miRNA regulators of glioblastoma aggressiveness. *Biomaterials, 107*, 74-87.

da Motta, L. L., De Bastiani, M. A., Stapenhorst, F., & Klamt, F. (2015). Oxidative stress associates with aggressiveness in lung large-cell carcinoma. *Tumor Biol., 36*, 4681-4688.

Dai, Z. K., Qin, J. K., Huang, J. E., Luo, Y., Xu, Q., & Zhao, H. L. (2012). Tanshinone IIA activates calcium-dependent apoptosis signaling pathway in human hepatoma cells. *J. Nat. Med., 66*, 192-201.

Datta, J., Majumder, S., Kutay, H., Motiwala, T., Frankel, W., Costa, R., Cha, H. C., MacDougald, O. A., Jacob, S. T., & Ghoshal, K. (2007). Metallothionein expression is

ACCEPTED MANUSCRIPT

suppressed in primary human hepatocellular carcinomas and is mediated through inactivation of CCAAT/enhancer binding protein alpha by phosphatidylinositol 3-kinase signaling cascade. *Cancer Res., 67*, 2736-2746.

Dawson, M. A., & Kouzarides, T. (2012). Cancer Epigenetics: From Mechanism to Therapy. *Cell, 150*, 12-27.

de Aquino, P. F., Carvalho, P. C., Nogueira, F. C. S., da Fonseca, C. O., Silva, J., Carvalho, M. D. D., Domont, G. B., Zanchin, N. I. T., & Fischer, J. D. D. (2016). A Time-Based and Intratumoral Proteomic Assessment of a Recurrent Glioblastoma Multiforme. *Front. Oncol., 6*, 1-10.

Delaney, J. R., & Stupack, D. G. (2016). Whole Genome Pathway Analysis Identifies an Association of Cadmium Response Gene Loss with Copy Number Variation in Mutant p53 Bearing Uterine Endometrial Carcinomas. *Plos One, 11*, 1-17.

Deng, D. J., El-Rifai, W., Ji, J. F., Zhu, B. D., Trampont, P., Li, J. Y., Smith, M. F., & Powel, S. M. (2003). Hypermethylation of metallothionein-3 CpG island in gastric carcinoma. *Carcinogenesis, 24*, 25-29.

Ding, J., & Lu, S. C. (2016). Low metallothionein 1M expression association with poor hepatocellular carcinoma prognosis after curative resection. *Genet. Mol. Res., 15*, 1-10.

Dong, X., Ding, W., Ye, J., Yan, D., Xue, F., Xu, L., Yin, J., & Guo, W. (2016). MiR-24-3p enhances cell growth in hepatocellular carcinoma by targeting metallothionein 1M. *Cell Biochem. Funct., 34*, 491-496.

Du, H. Y., Li, Y. H., Olivo, M., Yip, G. W. C., & Bay, B. H. (2006). Differential up-regulation of metallothionein isoforms in well-differentiated nasopharyngeal cancer cells in vitro by photoactivated hypericin. *Oncol. Rep., 16*, 1397-1402.

Dutton, M. D., Stephenson, M., & Klaverkamp, J. F. (1993). A Mercury saturation assay for measuring metallothionein in fish. *Environ. Toxicol. Chem., 12*, 1193-1202.

El Sharkawy, S. L., Abbas, N. F., Badawi, M. A., & El Shaer, M. A. (2006). Metallothionein isoform II expression in hyperplastic, dysplastic and neoplastic prostatic lesions. *J. Clin. Pathol., 59*, 1171-1174.

Emri, E., Egervari, K., Varvolgyi, T., Rozsa, D., Miko, E., Dezso, B., Veres, I., Mehes, G., Emri, G., & Remenyik, E. (2013). Correlation among metallothionein expression, intratumoural macrophage infiltration and the risk of metastasis in human cutaneous malignant melanoma. *J. Eur. Acad. Dermatol. Venereol., 27*, e320-e327.

Endo-Munoz, L., Cumming, A., Sommerville, S., Dickinson, I., & Saunders, N. A. (2010). Osteosarcoma is characterised by reduced expression of markers of osteoclastogenesis and antigen presentation compared with normal bone. *Br. J. Cancer, 103*, 73-81.

Esteller, M. (2007). Cancer epigenomics: DNA methylomes and histone-modification maps. *Nature Reviews Genetics, 8*, 286-298.

Fabbri, M., Urani, C., Sacco, M. G., Procaccianti, C., & Gribaldo, L. (2012). Whole genome analysis and microRNAs regulation in HepG2 cells exposed to cadmium. *ALTEX-Altern. Anim. Exp., 29*, 173-182.

Faller, W. J., Rafferty, M., Hegarty, S., Gremel, G., Ryan, D., Fraga, M. F., Esteller, M., Dervan, P. A., & Gallagher, W. M. (2010). Metallothionein 1E is methylated in malignant melanoma and increases sensitivity to cisplatin-induced apoptosis. *Melanoma Res., 20*, 392-400.

Falnoga, I., Pevec, A. Z., Slejkovec, Z., Znidaric, M. T., Zajc, I., Mlakar, S. J., & Marc, J. (2012). Arsenic Trioxide (ATO) Influences the Gene Expression of Metallothioneins in Human Glioblastoma Cells. *Biol. Trace Elem. Res., 149*, 331-339.

Fan, L. Z., & Cherian, M. G. (2002). Potential role of p53 on metallothionein induction in human epithelial breast cancer cells. *Br. J. Cancer, 87*, 1019-1026.

ACCEPTED MANUSCRIPT

Felizola, S. J. A., Nakamura, Y., Arata, Y., Ise, K., Satoh, F., Rainey, W. E., Midorikawa, S., Suzuki, S., & Sasano, H. (2014). Metallothionein-3 (MT-3) in the Human Adrenal Cortex and its Disorders. *Endocr. Pathol., 25*, 229-235.

Ferrario, C., Lavagni, P., Gariboldi, M., Miranda, C., Losa, M., Cleris, L., Formelli, F., Pilotti, S., Pierotti, M. A., & Greco, A. (2008). Metallothionein 1G acts as an oncosupressor in papillary thyroid carcinoma. *Lab. Invest., 88*, 474-481.

Forma, E., Krzeslak, A., Wilkosz, J., Jozwiak, P., Szymczyk, A., Rozanski, W., & Brys, M. (2012). Metallothionein 2A genetic polymorphisms and risk of prostate cancer in a Polish population. *Cancer Genet., 205*, 432-435.

Friedline, J. A., Garrett, S. H., Somji, S., Todd, J. H., & Sens, D. A. (1998). Differential expression of the MT-1E gene in estrogen-receptor-positive and -negative human breast cancer cell lines. *Am. J. Pathol., 152*, 23-27.

Fu, C. L., Pan, B., Pan, J. H., & Gan, M. F. (2017). Metallothionein 1M suppresses tumorigenesis in hepatocellular carcinoma. *Oncotarget, 8*, 33037-33046.

Fu, J., Lv, H. J., Guan, H. X., Ma, X. Y., Ji, M. J., He, N. Y., Shi, B. Y., & Hou, P. (2013). Metallothionein 1G functions as a tumor suppressor in thyroid cancer through modulating the PI3K/Akt signaling pathway. *BMC Cancer, 13*, 1-13.

Furi, I., Kalmar, A., Wichmann, B., Spisak, S., Scholler, A., Bartak, B., Tulassay, Z., & Molnar, B. (2015). Cell Free DNA of Tumor Origin Induces a 'Metastatic' Expression Profile in HT-29 Cancer Cell Line. *Plos One, 10*, 1-16.

Garrett, S. H., Sens, M. A., Shukla, D., Flores, L., Somji, S., Todd, J. H., & Sens, D. A. (2000). Metallothionein isoform 1 and 2 gene expression in the human prostate: Downregulation of MT-1X in advanced prostate cancer. *Prostate, 43*, 125-135.

Garrett, S. H., Sens, M. A., Shukla, D., Nestor, S., Somji, S., Todd, J. H., & Sens, D. A. (1999). Metallothionein isoform 3 expression in the human prostate and cancer-derived cell lines. *Prostate, 41*, 196-202.

Garrett, S. H., Somji, S., Todd, J. H., Sens, D. A., Lamm, D. L., & Sens, M. A. (1999). *Metallothionein isoform gene expression in four human bladder cancer cell lines*. Basel: Birkhauser Verlag Ag.

Gelfand, R., Vernet, D., Bruhn, K., Vadgama, J., & Gonzalez-Cadavid, N. F. (2016). Long-term exposure of MCF-12A normal human breast epithelial cells to ethanol induces epithelial mesenchymal transition and oncogenic features. *Int. J. Oncol., 48*, 2399-2414.

Gelfand, R., Vernet, D., Bruhn, K. W., Sarkissyan, S., Heber, D., Vadgama, J. V., & Gonzalez-Cadavid, N. F. (2017). Long-term exposure of MCF-7 breast cancer cells to ethanol stimulates oncogenic features. *Int. J. Oncol., 50*, 49-65.

Ghoshal, K., Majumder, S., Li, Z. L., Dong, X. C., & Jacob, S. T. (2000). Suppression of metallothionein gene expression in a rat hepatoma because of promoter-specific DNA methylation. *J. Biol. Chem., 275*, 539-547.

Giacomini, C. P., Leung, S. Y., Chen, X., Yuen, S. T., Kim, Y. H., Bair, E., & Pollack, J. R. (2005). A gene expression signature of genetic instability in colon cancer. *Cancer Res., 65*, 9200-9205.

Girnun, G. D., Naseri, E., Vafai, S. B., Qu, L., Szwaya, J. D., Bronson, R., Alberta, J. A., & Spiegelman, B. M. (2007). Synergy between PPAR gamma ligands and platinum-based drugs in cancer. *Cancer Cell, 11*, 395-406.

Gomulkiewicz, A., Jablonska, K., Pula, B., Grzegrzolka, J., Borska, S., Podhorska-Okolow, M., Wojnar, A., Rys, J., Ambicka, A., Ugorski, M., Zabel, M., & Dziegiel, P. (2016). Expression of metallothionein 3 in ductal breast cancer. *Int. J. Oncol., 49*, 2487-2497.

ACCEPTED MANUSCRIPT

Gondo, Y., Satsu, H., Ishimoto, Y., Iwamoto, T., & Shimizu, M. (2012). Effect of taurine on mRNA expression of thioredoxin interacting protein in Caco-2 cells. *Biochem. Biophys. Res. Commun., 426*, 433-437.

Gosland, M., Lum, B., Schimmelpfennig, J., Baker, J., & Doukas, M. (1996). Insights into mechanisms of cisplatin resistance and potential for its clinical reversal. *Pharmacotherapy, 16*, 16-39.

Gumulec, J., Raudenska, M., Adam, V., Kizek, R., & Masarik, M. (2014). Metallothionein - Immunohistochemical Cancer Biomarker: A Meta-Analysis. *Plos One, 9*, 1-14.

Guo, R. L., Wu, G. M., Li, H. D., Qian, P., Han, J., Pan, F., Li, W. B., Li, J., & Ji, F. Y. (2013). Promoter Methylation Profiles between Human Lung Adenocarcinoma Multidrug Resistant A549/Cisplatin (A549/DDP) Cells and Its Progenitor A549 Cells. *Biol. Pharm. Bull., 36*, 1310-1316.

Gurel, V., Sens, D. A., Somji, S., Garrett, S. H., Weiland, T., & Sens, M. A. (2005). Post-transcriptional regulation of metallothionein isoform 1 and 2 expression in the human breast and the MCF-10A cell line. *Toxicol. Sci., 85*, 906-915.

Guschanski, K., Warnefors, M., & Kaessmann, H. (2017). The evolution of duplicate gene expression in mammalian organs. *Genome Res., 27*, 1461-1474.

Habel, N., Hamidouche, Z., Girault, I., Patino-Garcia, A., Lecanda, F., Marie, P. J., & Fromigue, O. (2013). Zinc chelation: a metallothionein 2A's mechanism of action involved in osteosarcoma cell death and chemotherapy resistance. *Cell Death Dis., 4*, 1-10.

Han, Y. C., Zheng, Z. L., Zuo, Z. H., Yu, Y. P., Chen, R., Tseng, G. C., Nelson, J. B., & Luo, J. H. (2013). Metallothionein 1h tumour suppressor activity in prostate cancer is mediated by euchromatin methyltransferase 1. *J. Pathol., 230*, 184-193.

Hanada, K., Sawamura, D., Hashimoto, I., Kida, K., & Naganuma, A. (1998). Epidermal proliferation of the skin in metallothionein-null mice. *J. Invest. Dermatol., 110*, 259-262.

Haq, F., Mahoney, M., & Koropatnick, J. (2003). Signaling events for metallothionein induction. *Mutat. Res.-Fundam. Mol. Mech. Mutagen., 533*, 211-226.

Hasumi, M., Suzuki, K., Matsui, H., Koike, H., Ito, K., & Yamanaka, H. (2003). Regulation of metallothionein and zinc transporter expression in human prostate cancer cells and tissues. *Cancer Lett., 200*, 187-195.

He, C. Y., Jiang, S. W., Jin, H. J., Chen, S. Z., Lin, G., Yao, H., Wang, X. Y., Mi, P., Ji, Z. L., Lin, Y. C., Lin, Z. N., & Liu, G. (2016). Mitochondrial electron transport chain identified as a novel molecular target of SPIO nanoparticles mediated cancer-specific cytotoxicity. *Biomaterials, 83*, 102-114.

Heger, Z., Rodrigo, M. A. M., Krizkova, S., Ruttkay-Nedecky, B., Zalewska, M., del Pozo, E. M. P., Pelfrene, A., Pourrut, B., Stiborova, M., Eckschlager, T., Emri, G., Kizek, R., & Adam, V. (2016). Metallothionein as a Scavenger of Free Radicals - New Cardioprotective Therapeutic Agent or Initiator of Tumor Chemoresistance? *Curr. Drug Targets, 17*, 1438-1451.

Henrique, R., Jeronimo, C., Hoque, M. O., Nomoto, S., Carvalho, A. L., Costa, V. L., Oliveira, J., Teixeira, M. R., Lopes, C., & Sidransky, D. (2005). MT1G hypermethylation is associated with higher tumor stage in prostate cancer. *Cancer Epidemiol. Biomarkers Prev., 14*, 1274-1278.

Hinkel, A., Schmidtchen, S., Palisaar, R. J., Noldus, J., & Pannek, J. (2008). Identification of bladder cancer patients at risk for recurrence or progression: An immunohistochemical study based on the expression of metallothionein. *Journal of Toxicology and Environmental Health-Part a-Current Issues, 71*, 954-959.

ACCEPTED MANUSCRIPT

Hou, X. F., Fan, Q. X., Wang, L. X., & Lu, S. X. (2009). Role of metallothionein1h in cisplatin resistance of non-small cell lung cancer cells. *Chin. J. Cancer Res., 21*, 247-254.

Houessinon, A., Franois, C., Sauzay, C., Louandre, C., Mongelard, G., Godin, C., Bodeau, S., Takahashi, S., Saidak, Z., Gutierrez, L., Regimbeau, J. M., Barget, N., Barbare, J. C., Ganne, N., Chauffert, B., Coriat, R., & Galmiche, A. (2016). Metallothionein-1 as a biomarker of altered redox metabolism in hepatocellular carcinoma cells exposed to sorafenib. *Mol. Cancer, 15*, 1-10.

Huang, Y., De La Chapelle, A., & Pellegata, N. S. (2003). Hypermethylation, but not LOH, is associated with the low expression of MT1G and CRABP1 in papillary thyroid carcinoma. *Int. J. Cancer, 104*, 735-744.

Hutt, J. A., Vuillemenot, B. R., Barr, E. B., Grimes, M. J., Hahn, F. F., Hobbs, C. H., March, T. H., Gigliotti, A. P., Seilkop, S. K., Finch, G. L., Mauderly, J. L., & Belinsky, S. A. (2005). Life-span inhalation exposure to mainstream cigarette smoke induces lung cancer in B6C3F1 mice through genetic and epigenetic pathways. *Carcinogenesis, 26*, 1999-2009.

Chan, H. M., Pringle, G. A., & Cherian, M. G. (1992). Heterogeneity of antibodies to metallothionein isomers and development of a simple enzyme-linked immunosorbent assay. *J. Biochem. Toxicol., 7*, 219-227.

Chan, K. Y. Y., Lai, P. B. S., Squire, J. A., Beheshti, B., Wong, N. L. Y., Sy, S. M. H., & Wong, N. (2006). Positional expression profiling indicates candidate genes in deletion hotspots of hepatocellular carcinoma. *Mod. Pathol., 19*, 1546-1554.

Chandler, P., Kochupurakkal, B. S., Alam, S., Richardson, A. L., Soybel, D. I., & Kelleher, S. L. (2016). Subtype-specific accumulation of intracellular zinc pools is associated with the malignant phenotype in breast cancer. *Mol. Cancer, 15*, 1-19.

Chao, C. C. K. (1996). Molecular basis of cis-diamminedichloroplatinum(II) resistance: A review. *Journal of the Formosan Medical Association, 95*, 893-900.

Cheng, T. C., Manorek, G., Samimi, G., Lin, X. J., Berry, C. C., & Howell, S. B. (2006). Identification of genes whose expression is associated with cisplatin resistance in human ovarian carcinoma cells. *Cancer Chemother. Pharmacol., 58*, 384-395.

Cherian, M. G., Jayasurya, A., & Bay, B. H. (2003). Metallothioneins in human tumors and potential roles in carcinogenesis. *Mutat. Res.-Fundam. Mol. Mech. Mutagen., 533*, 201-209.

Choi, J. K., Yu, U. S., Yoo, O. J., & Kim, S. (2005). Differential coexpression analysis using microarray data and its application to human cancer. *Bioinformatics, 21*, 4348-4355.

Chuang, Y. Y. E., Chen, Y. D., Chandramouli, G. V. R., Cook, J. A., Coffin, D., Tsai, M. H., DeGraff, W., Yan, H. L., Zhao, S. P., Russo, A., Liu, E. T., & Mitchell, J. B. (2002). Gene expression after treatment with hydrogen peroxide, menadione, or t-butyl hydroperoxide in breast cancer cells. *Cancer Res., 62*, 6246-6254.

Chung, M. J., Kang, A. Y., Lee, K. M., Oh, E., Jun, H. J., Kim, S. Y., Auh, J. H., Moon, T. W., Lee, S. J., & Park, K. H. (2006). Water-soluble genistin glycoside isoflavones up-regulate antioxidant metallothionein expression and scavenge free radicals. *J. Agric. Food Chem., 54*, 3819-3826.

Iljin, K., Ketola, K., Vainio, P., Halonen, P., Kohonen, P., Fey, V., Grafstrom, R. C., Perala, M., & Kallioniemi, O. (2009). High-Throughput Cell-Based Screening of 4910 Known Drugs and Drug-like Small Molecules Identifies Disulfiram as an Inhibitor of Prostate Cancer Cell Growth. *Clin. Cancer Res., 15*, 6070-6078.

Jadhav, R. R., Ye, Z. Q., Huang, R. L., Liu, J., Hsu, P. Y., Huang, Y. W., Rangel, L. B., Lai, H. C., Roa, J. C., Kirma, N. B., Huang, T. H. M., & Jin, V. X. (2015). Genome-wide

ACCEPTED MANUSCRIPT

DNA methylation analysis reveals estrogen-mediated epigenetic repression of metallothionein-1 gene cluster in breast cancer. *Clin. Epigenetics, 7*, 1-15.

Jansova, E., Koutna, I., Krontorad, P., Svoboda, Z., Krivankova, S., Zaloudik, J., Kozubek, M., & Kozubek, S. (2006). Comparative transcriptome maps: a new approach to the diagnosis of colorectal carcinoma patients using cDNA microarrays. *Clin. Genet., 69*, 218-227.

Janssen, A. M. L., van Duijn, W., Oostendorp-van de Ruit, M. M., Kruidenier, L., Bosman, C. B., Griffioen, G., Lamers, C., van Krieken, J., van de Velde, C. J. H., & Verspaget, H. W. (2000). Metallothionein in human gastrointestinal cancer. *J. Pathol., 192*, 293-300.

Jason, T. L. H., Koropatnick, J., & Berg, R. W. (2004). Toxicology of antisense therapeutics. *Toxicol. Appl. Pharmacol., 201*, 66-83.

Jeong, H. G., Youn, C. K., Cho, H. J., Kim, S. H., Kim, M. H., Kim, H. B., Chang, I. Y., Lee, Y. S., Chung, M. H., & You, H. J. (2004). Metallothionein-III prevents gamma-ray-induced 8-oxoguanine accumulation in normal and hOGG1-depleted cells. *J. Biol. Chem., 279*, 34138-34149.

Ji, X. F., Fan, Y. C., Gao, S., Yang, Y., Zhang, J. J., & Wang, K. (2014). MT1M and MT1G promoter methylation as biomarkers for hepatocellular carcinoma. *World J. Gastroenterol., 20*, 4723-4729.

Jin, H. Y., Tan, X. Z., Liu, X. F., & Ding, Y. J. (2010). The study of effect of tea polyphenols on microsatellite instability colorectal cancer and its molecular mechanism. *Int. J. Colorectal Dis., 25*, 1407-1415.

Jin, R., Bay, B. H., Chow, V. T. K., Tan, P. H., & Lin, V. C. L. (2000). Metallothionein 1E mRNA is highly expressed in oestrogen receptor-negative human invasive ductal breast cancer. *Br. J. Cancer, 83*, 319-323.

Jin, R. X., Bay, B. H., Chow, V. T. K., & Tan, P. H. (2001). Metallothionein 1F mRNA expression correlates with histological grade in breast carcinoma. *Breast Cancer Res. Treat., 66*, 265-272.

Jin, R. X., Bay, B. H., Chow, V. T. K., Tan, P. H., & Dheen, T. (2001). Significance of metallothionein expression in breast myoepithelial cells. *Cell Tissue Res., 303*, 221-226.

Jin, R. X., Chow, V. T. K., Tan, P. H., Dheen, S. T., Duan, W., & Bay, B. H. (2002). Metallothionein 2A expression is associated with cell proliferation in breast cancer. *Carcinogenesis, 23*, 81-86.

Jogi, A., Vallon-Christersson, J., Holmquist, L., Axelson, H., Borg, A., & Pahlman, S. (2004). Human neuroblastoma cells exposed to hypoxia: induction of genes associated with growth, survival, and aggressive behavior. *Exp. Cell Res., 295*, 469-487.

Juang, H. H., Chung, L. C., Sung, H. C., Feng, T. H., Lee, Y. H., Chang, P. L., & Tsui, K. H. (2013). Metallothionein 3: An androgen-upregulated gene enhances cell invasion and tumorigenesis of prostate carcinoma cells. *Prostate, 73*, 1495-1506.

Kanda, M., Nomoto, S., Okamura, Y., Nishikawa, Y., Sugimoto, H., Kanazumi, N., Takeda, S., & Nakao, A. (2009). Detection of metallothionein 1G as a methylated tumor suppressor gene in human hepatocellular carcinoma using a novel method of double combination array analysis. *Int. J. Oncol., 35*, 477-483.

Kennette, W., Collins, O. M., Zalups, R. K., & Koropatnick, J. (2005). Basal and zinc-induced metallothionein in resistance to cadmium, cisplatin, zinc, and tertButyl hydroperoxide: Studies using MT knockout and antisense-downregulated MT in mammalian cells. *Toxicol. Sci., 88*, 602-613.

Kim, H. G., Kim, J. Y., Han, E. H., Hwang, Y. P., Choi, J. H., Park, B. H., & Jeong, H. G. (2011). Metallothionein-2A overexpression increases the expression of matrix metalloproteinase-9 and invasion of breast cancer cells. *FEBS Lett., 585*, 421-428.

ACCEPTED MANUSCRIPT

Kim, J. M., Sohn, H. Y., Yoon, S. Y., Oh, J. H., Yang, J. O., Kim, J. H., Song, K. S., Rho, S. M., Yoo, H. S., Kim, Y. S., Kim, J. G., & Kim, N. S. (2005). Identification of gastric cancer-related genes using a cDNA microarray containing novel expressed sequence tags expressed in gastric cancer cells. *Clin. Cancer Res., 11*, 473-482.

Kim, T. R., Lee, H. M., Lee, S. Y., Kim, E. J., Kim, K. C., Paik, S. G., Cho, E. W., & Kim, I. G. (2010). SM22 alpha-induced activation of p16(INK4a)/retinoblastoma pathway promotes cellular senescence caused by a subclinical dose of gamma-radiation and doxorubicin in HepG2 cells. *Biochem. Biophys. Res. Commun., 400*, 100-105.

Klaassen, C. D., Liu, J., & Diwan, B. A. (2009). Metallothionein protection of cadmium toxicity. *Toxicol. Appl. Pharmacol., 238*, 215-220.

Klutstein, M., Nejman, D., Greenfield, R., & Cedar, H. (2016). DNA Methylation in Cancer and Aging. *Cancer Res., 76*, 3446-3450.

Kmiecik, A. M., Pula, B., Suchanski, J., Olbromski, M., Gomulkiewicz, A., Owczarek, T., Kruczak, A., Ambicka, A., Rys, J., Ugorski, M., Podhorska-Okolow, M., & Dziegiel, P. (2015). Metallothionein-3 Increases Triple-Negative Breast Cancer Cell Invasiveness via Induction of Metalloproteinase Expression. *Plos One, 10*, 1-25.

Koga, Y., Pelizzola, M., Cheng, E., Krauthammer, M., Sznol, M., Ariyan, S., Narayan, D., Molinaro, A. M., Halaban, R., & Weissman, S. M. (2009). Genome-wide screen of promoter methylation identifies novel markers in melanoma. *Genome Res., 19*, 1462-1470.

Krizkova, S., Blahova, P., Nakielna, J., Fabrik, I., Adam, V., Eckschlager, T., Beklova, M., Svobodova, Z., Horak, V., & Kizek, R. (2009). Comparison of Metallothionein Detection by Using Brdicka Reaction and Enzyme-Linked Immunosorbent Assay Employing Chicken Yolk Antibodies. *Electroanalysis, 21*, 2575-2583.

Krizkova, S., Kepinska, M., Emri, G., Rodrigo, M. A. M., Tmejova, K., Nerudova, D., Kizek, R., & Adam, V. (2016). Microarray analysis of metallothioneins in human diseases-A review. *J. Pharm. Biomed. Anal., 117*, 464-473.

Krizkova, S., Ryvolova, M., Hrabeta, J., Adam, V., Stiborova, M., Eckschlager, T., & Kizek, R. (2012). Metallothioneins and zinc in cancer diagnosis and therapy. *Drug Metab. Rev., 44*, 287-301.

Krzeslak, A., Forma, E., Chwatko, G., Jozwiak, P., Szymczyk, A., Wilkosz, J., Rozanski, W., & Brys, M. (2013). Effect of metallothionein 2A gene polymorphism on allele-specific gene expression and metal content in prostate cancer. *Toxicol. Appl. Pharmacol., 268*, 278-285.

Krzeslak, A., Forma, E., Jozwiak, P., Szymczyk, A., Smolarz, B., Romanowicz-Makowska, H., Rozanski, W., & Brys, M. (2014). Metallothionein 2A genetic polymorphisms and risk of ductal breast cancer. *Clin. Exper. Med., 14*, 107-113.

Kumar, A., Chatopadhyay, T., Raziuddin, M., & Ralhan, R. (2007). Discovery of deregulation of zinc homeostasis and its associated genes in esophageal squamous cell carcinoma using cDNA microarray. *Int. J. Cancer, 120*, 230-242.

Kwabi-Addo, B., Wang, S. P., Chung, W., Jelinek, J., Patierno, S. R., Wang, B. D., Andrawis, R., Lee, N. H., Apprey, V., Issa, J. P., & Ittmann, M. (2010). Identification of Differentially Methylated Genes in Normal Prostate Tissues from African American and Caucasian Men. *Clin. Cancer Res., 16*, 3539-3547.

Lai, Y. Y., Lim, D. N., Tan, P. H., Leung, T. K. C., Yip, G. W. C., & Bay, B. H. (2010). Silencing the Metallothionein-2A Gene Induces Entosis in Adherent MCF-7 Breast Cancer Cells. *Anat. Rec., 293*, 1685-1691.

Lai, Y. Y., Yip, G. W. C., & Bay, B. H. (2011). Targeting Metallothionein for Prognosis and Treatment of Breast Cancer. *Recent Patents Anti-Canc. Drug Discov., 6*, 178-185.

ACCEPTED MANUSCRIPT

Lee, J. D., Wu, S. M., Lu, L. Y., Yang, Y. T., & Jeng, S. Y. (2009). Cadmium concentration and metallothionein expression in prostate cancer and benign prostatic hyperplasia of humans. *Journal of the Formosan Medical Association, 108*, 554-559.

Lee, J. H., Chae, J. W., Kim, J. K., Kim, H. J., Chung, J. Y., & Kim, Y. H. (2015). Inhibition of cisplatin-resistance by RNA interference targeting metallothionein using reducible oligo-peptoplex. *J. Control. Release, 215*, 82-90.

Lee, R. C., Feinbaum, R. L., & Ambros, V. (1993). The C-Elegands heterochronic gene Lin-4 encodes small RNAs with antisense complemetarity to Lin-14. *Cell, 75*, 843-854.

Lee, Y. C., Wang, H. P., Wang, C. P., Ko, J. Y., Lee, J. M., Chiu, H. M., Lin, J. T., Yamashita, S., Oka, D., Watanabe, N., Matsuda, Y., Ushijima, T., & Wu, M. S. (2011). Revisit of Field Cancerization in Squamous Cell Carcinoma of Upper Aerodigestive Tract: Better Risk Assessment with Epigenetic Markers. *Cancer Prev. Res., 4*, 1982-1992.

Li, H., Lu, Y. F., Chen, H., & Liu, J. (2017). Dysregulation of metallothionein and circadian genes in human hepatocellular carcinoma. *Chronobiol. Int., 34*, 192-202.

Li, K., Prow, T., Lemon, S. M., & Beard, M. R. (2002). Cellular response to conditional expression of hepatitis C virus core protein in Huh7 cultured human hepatoma cells. *Hepatology, 35*, 1237-1246.

Liang, G. Y., Lu, S. X., Xu, G., Liu, X. D., Li, J., & Zhang, D. S. (2013). Expression of metallothionein and Nrf2 pathway genes in lung cancer and cancer-surrounding tissues. *World J. Surg. Oncol., 11*, 1-5.

Lim, D., Jocelyn, K. M. X., Yip, G. W. C., & Bay, B. H. (2009). Silencing the Metallothionein-2A gene inhibits cell cycle progression from G1-to S-phase involving ATM and cdc25A signaling in breast cancer cells. *Cancer Lett., 276*, 109-117.

Lin, S. F., Wei, H., Maeder, D., Franklin, R. B., & Feng, P. (2009). Profiling of zinc-altered gene expression in human prostate normal vs. cancer cells: a time course study. *J. Nutr. Biochem., 20*, 1000-1012.

Liu, J., Lian, Z., Han, S., Waye, M. M. Y., Wang, H., Wu, M. C., Wu, K., Ding, J., Arbuthnot, P., Kew, M., Fan, D., & Feitelson, M. A. (2006). Downregulation of E-cadherin by hepatitis B virus X antigen in hepatocellullar carcinoma. *Oncogene, 25*, 1008-1017.

Liu, T., Bauskin, A. R., Zaunders, J., Brown, D. A., Pankurst, S., Russell, P. J., & Breit, S. N. (2003). Macrophage inhibitory cytokine 1 reduces cell adhesion and induces apoptosis in prostate cancer cells. *Cancer Res., 63*, 5034-5040.

Liu, Y., Liu, L., Yu, T., Lin, H. C., Chu, D., Deng, W., Yan, M. X., Li, J., & Yao, M. (2016). Systematic analysis of mRNA expression profiles in NSCLC cell lines to screen metastasis-related genes. *Mol. Med. Rep., 14*, 5093-5103.

Liu, Z. M., van Hasselt, C. A., Song, F. Z., Vlantis, A. C., Cherian, M. G., Koropatnick, J., & Chen, G. G. (2009). Expression of functional metallothionein isoforms in papillary thyroid cancer. *Mol. Cell. Endocrinol., 302*, 92-98.

Liuzzi, J. P., & Yoo, C. W. (2013). Role of Zinc in the Regulation of Autophagy During Ethanol Exposure in Human Hepatoma Cells. *Biol. Trace Elem. Res., 156*, 350-356.

Louhelainen, J. P., Hurst, C. D., Pitt, E., Nishiyama, H., Pickett, H. A., & Knowles, M. A. (2006). DBC1 re-expression alters the expression of multiple components of the plasminogen pathway. *Oncogene, 25*, 2409-2419.

Lu, J., Getz, G., Miska, E. A., Alvarez-Saavedra, E., Lamb, J., Peck, D., Sweet-Cordero, A., Ebet, B. L., Mak, R. H., Ferrando, A. A., Downing, J. R., Jacks, T., Horvitz, H. R., & Golub, T. R. (2005). MicroRNA expression profiles classify human cancers. *Nature, 435*, 834-838.

ACCEPTED MANUSCRIPT

Ma, D., Zhou, Z., Yang, B., He, Q., Zhang, Q., & Zhang, X. H. (2015). Association of molecular biomarkers expression with biochemical recurrence in prostate cancer through tissue microarray immunostaining. *Oncol. Lett., 10*, 2185-2191.

Magda, D., Lecane, P., Miller, R. A., Lepp, C., Miles, D., Mesfin, M., Biaglow, J. E., Ho, V. V., Chawannakul, D., Nagpal, S., Karaman, M. W., & Hacia, J. G. (2005). Motexafin gadolinium disrupts zinc metabolism in human cancer cell lines. *Cancer Res., 65*, 3837-3845.

Majumder, S., Kutay, H., Datta, J., Summers, D., Jacob, S. T., & Ghoshal, K. (2006). Epigenetic regulation of metallothionein-1 gene expression: Differential regulation of methylated and unmethylated promoters by DNA methyltransferases and methyl CpG binding proteins. *J. Cell. Biochem., 97*, 1300-1316.

Mao, J., Yu, H. X., Wang, C. J., Sun, L. H., Jiang, W., Zhang, P. Z., Xiao, Q. Y., Han, D. B., Saiyin, H., Zhu, J. D., Chen, T. Y., Roberts, L. R., Huang, H. J., & Yu, L. (2012). Metallothionein MT1M is a tumor suppressor of human hepatocellular carcinomas. *Carcinogenesis, 33*, 2568-2577.

Mao, P., Hever, M. P., Niemaszyk, L. M., Haghkerdar, J. M., Yanco, E. G., Desai, D., Beyrouthy, M. J., Kerley-Hamilton, J. S., Freemantle, S. J., & Spinella, M. J. (2011). Serine/Threonine Kinase 17A Is a Novel p53 Target Gene and Modulator of Cisplatin Toxicity and Reactive Oxygen Species in Testicular Cancer Cells. *J. Biol. Chem., 286*, 19381-19391.

Mehrian-Shai, R., Yalon, M., Simon, A. J., Eyal, E., Pismenyuk, T., Moshe, I., Constantini, S., & Toren, A. (2015). High metallothionein predicts poor survival in glioblastoma multiforme. *BMC Med. Genomics, 8*, 1-9.

Mensah, A. A., Kwee, I., Gaudio, E., Rinaldi, A., Ponzoni, M., Cascione, L., Fossati, G., Stathis, A., Zucca, E., Caprini, G., & Bertoni, F. (2015). Novel HDAC inhibitors exhibit pre-clinical efficacy in lymphoma models and point to the importance of CDKN1A expression levels in mediating their anti-tumor response. *Oncotarget, 6*, 5059-5071.

Miura, N., & Koizumi, S. (2007). Heavy metal responses of the human metallothionein isoform genes. *Yakugaku Zasshi-J. Pharm. Soc. Jpn., 127*, 665-673.

Moleirinho, A., Carneiro, J., Matthiesen, R., Silva, R. M., Amorim, A., & Azevedo, L. (2011). Gains, Losses and Changes of Function after Gene Duplication: Study of the Metallothionein Family. *Plos One, 6*, 1-9.

Morandi, L., de Biase, D., Visani, M., Monzoni, A., Tosi, A., Brulatti, M., Turchetti, D., Baccarini, P., Tallini, G., & Pession, A. (2012). T- 20 repeat in the 3 '-untranslated region of the MT1X gene: a marker with high sensitivity and specificity to detect microsatellite instability in colorectal cancer. *Int. J. Colorectal Dis., 27*, 647-656.

Mougeot, J. L. C., Bahrani-Mostafavi, Z., Vachris, J. C., McKinney, K. Q., Gurlov, S., Zhang, J., Naumann, R. W., Higgins, R. V., & Hall, J. B. (2006). Gene expression profiling of ovarian tissues for determination of molecular pathways reflective of tumorigenesis. *J. Mol. Biol., 358*, 310-329.

Mounicou, S., Ouerdane, L., L'Azou, B., Passagne, I., Ohayon-Courtes, C., Szpunar, J., & Lobinski, R. (2010). Identification of Metallothionein Subisoforms in HPLC Using Accurate Mass and Online Sequencing by Electrospray Hybrid Linear Ion Trap-Orbital Ion Trap Mass Spectrometry. *Anal. Chem., 82*, 6947-6957.

Naito, S., Yokomizo, A., & Koga, H. (1999). Mechanisms of drug resistance in chemotherapy for urogenital carcinoma. *International Journal of Urology, 6*, 427-439.

Nakamura, H., Wang, Y. W., Xue, H., Romanish, M. T., Mager, D. L., Helgason, C. D., & Wang, Y. Z. (2013). Genistein versus ICI 182, 780: An Ally or Enemy in Metastatic Progression of Prostate Cancer. *Prostate, 73*, 1747-1760.

ACCEPTED MANUSCRIPT

Nakane, H., Hirano, M., Ito, H., Hosono, S., Oze, I., Matsuda, F., Tanaka, H., & Matsuo, K. (2015). Impact of metallothionein gene polymorphisms on the risk of lung cancer in a Japanese population. *Mol. Carcinog., 54*, E122-E128.

Nguyen, A., Jing, Z., Mahoney, P. S., Davis, R., Sikka, S. C., Agrawal, K. C., & Abdel-Mageed, A. B. (2000). In vivo gene expression profile analysis of metallothionein in renal cell carcinoma. *Cancer Lett., 160*, 133-140.

Niwa, T., Tsukamoto, T., Toyoda, T., Mori, A., Tanaka, H., Maekita, T., Ichinose, M., Tatematsu, M., & Ushijima, T. (2010). Inflammatory Processes Triggered by Helicobacter pylori Infection Cause Aberrant DNA Methylation in Gastric Epithelial Cells. *Cancer Res., 70*, 1430-1440.

Nixon, J. B., Kim, K. S., Lamb, P. W., Bottone, F. G., & Eling, T. E. (2004). 15-lipoxygenase-1 has anti-tumorigenic effects in colorectal cancer. *Prostaglandins Leukot. Essent. Fatty Acids, 70*, 7-15.

Norris, J. L., & Caprioli, R. M. (2013). Analysis of Tissue Specimens by Matrix-Assisted Laser Desorption/Ionization Imaging Mass Spectrometry in Biological and Clinical Research. *Chem. Rev., 113*, 2309-2342.

Nylund, C., Rappu, P., Pakula, E., Heino, A., Laato, L., Elo, L. L., Vihinen, P., Pyrhonen, S., Owen, G. R., Larjava, H., Kallajoki, M., & Heino, J. (2012). Melanoma-Associated Cancer-Testis Antigen 16 (CT16) Regulates the Expression of Apoptotic and Antiapoptotic Genes and Promotes Cell Survival. *Plos One, 7*, 1-12.

Ogra, Y., & Suzuki, K. T. (1999). Biological significance of non-acetylated metallothionein. *J. Chromatogr. B, 735*, 17-24.

Oka, D., Yamashita, S., Tomioka, T., Nakanishi, Y., Kato, H., Kaminishi, M., & Ushijima, T. (2009). The Presence of Aberrant DNA Methylation in Noncancerous Esophageal Mucosae in Association With Smoking History A Target for Risk Diagnosis and Prevention of Esophageal Cancers. *Cancer, 115*, 3412-3426.

Olszewski, U., Claffey, J., Hogan, M., Tacke, M., Zeillinger, R., Bednarski, P. J., & Hamilton, G. (2011). Anticancer activity and mode of action of titanocene C. *Invest. New Drugs, 29*, 607-614.

Orbo, A., Moe, B. T., Gronaas, H., & Paulssen, R. H. (2009). Early effects of high concentrations of progesterone and Mifepristone A gene expression study of endometrial cancer cells (Ishikawa). *J. Steroid Biochem. Mol. Biol., 113*, 139-149.

Ostrakhovitch, E. A., Olsson, P. E., von Hofsten, J., & Cherian, M. G. (2007). P53 mediated regulation of metallothionein transcription in breast cancer cells. *J. Cell. Biochem., 102*, 1571-1583.

Ostrakhovitch, E. A., Song, Y. P., & Cherian, M. G. (2016). Basal and copper-induced expression of metallothionein isoform 1,2 and 3 genes in epithelial cancer cells: The role of tumor suppressor p53. *J. Trace Elem. Med. Biol., 35*, 18-29.

Pan, Y. M., Huang, J. Q., Xing, R., Yin, X., Cui, J. T., Li, W. M., Yu, J., & Lu, Y. Y. (2013). Metallothionein 2A inhibits NF-kappa B pathway activation and predicts clinical outcome segregated with TNM stage in gastric cancer patients following radical resection. *J. Transl. Med., 11*, 1-14.

Pan, Y. M., Lin, S. Y., Xing, R., Zhu, M., Lin, B. N., Cui, J. T., Li, W. M., Gao, J., Shen, L., Zhao, Y. Y., Guo, M. Z., Wang, J. M., Huang, J. Q., & Lu, Y. Y. (2016). Epigenetic Upregulation of Metallothionein 2A by Diallyl Trisulfide Enhances Chemosensitivity of Human Gastric Cancer Cells to Docetaxel Through Attenuating NF-B Activation. *Antioxid. Redox Signal., 24*, 839-854.

Pan, Y. M., Xing, R., Cui, J. T., Li, W. M., & Lu, Y. Y. (2013). Clinicopathological significance of altered metallothionein 2A expression in gastric cancer according to Lauren's classification. *Chin. Med. J., 126*, 2681-2686.

ACCEPTED MANUSCRIPT

Panderi, I., Yakirevich, E., Papagerakis, S., Noble, L., Lombardo, K., & Pantazatos, D. (2017). Differentiating tumor heterogeneity in formalin-fixed paraffin-embedded (FFPE) prostate adenocarcinoma tissues using principal component analysis of matrix-assisted laser desorption/ionization imaging mass spectral data. *Rapid Commun. Mass Spectrom., 31*, 160-170.

Paulssen, R. H., Moe, B., Gronaas, H., & Orbo, A. (2008). Gene expression in endometrial cancer cells (Ishikawa) after short time high dose exposure to progesterone. *Steroids, 73*, 116-128.

Pedersen, M. O., Larsen, A., Stoltenberg, M., & Penkowa, M. (2009). The role of metallothionein in oncogenesis and cancer prognosis. *Prog. Histochem. Cytochem., 44*, 29-64.

Peng, B., Gu, Y. X., Xiong, Y., Zheng, G. P., & He, Z. M. (2012). Microarray-Assisted Pathway Analysis Identifies MT1X & NF kappa B as Mediators of TCRP1-Associated Resistance to Cisplatin in Oral Squamous Cell Carcinoma. *Plos One, 7*, 1-13.

Peng, D. F., Hu, T. L., Jiang, A. X., Washington, M. K., Moskaluk, C. A., Schneider-Stock, R., & El-Rifai, W. (2011). Location-Specific Epigenetic Regulation of the Metallothionein 3 Gene in Esophageal Adenocarcinomas. *Plos One, 6*, 1-10.

Perez, R. P. (1998). Cellular and molecular determinants of cisplatin resistance. *European Journal of Cancer, 34*, 1535-1542.

Peyre, M., Commo, F., Dantas-Barbosa, C., Andreiuolo, F., Puget, S., Lacroix, L., Drusch, F., Scott, V., Varlet, P., Mauguen, A., Dessen, P., Lazar, V., Vassal, G., & Grill, J. (2010). Portrait of ependymoma recurrence in children: biomarkers of tumor progression identified by dual-color microarray-based gene expression analysis. *Plos One, 5*, 1-15.

Piotrowski, A., Benetkiewicz, M., Menzel, U., de Stahl, T. D., Mantripragada, K., Grigelionis, G., Buckley, P. G., Jankowski, M., Hoffman, J., Bala, D., Srutek, E., Laskowski, R., Zegarski, W., & Dumanski, J. P. (2006). Microarray-based survey of CpG islands identifies concurrent hyper- and hypomethylation patterns in tissues derived from patients with breast cancer. *Gene Chromosomes Cancer, 45*, 656-667.

Pontes, H. A. R., Xavier, F. C. D., da Silva, T. S. P., Fonseca, F. P., Paiva, H. B., Pontes, F. S. C., & Pinto, D. D. (2009). Metallothionein and p-Akt proteins in oral dysplasia and in oral squamous cell carcinoma: an immunohistochemical study. *Journal of Oral Pathology & Medicine, 38*, 644-650.

Poulsen, C. B., Borup, R., Borregaard, N., Nielsen, F. C., Moller, M. B., & Ralfkiaer, E. (2006). Prognostic significance of metallothionein in B-cell lymphomas. *Blood, 108*, 3514-3519.

Prasmickaite, L., Cekaite, L., Hellum, M., Hovig, E., Hogset, A., & Berg, K. (2006). Transcriptome changes in a colon adenocarcinoma cell line in response to photochemical treatment as used in photochemical internalisation (PCI). *FEBS Lett., 580*, 5739-5746.

Prokopczyk, B., Sinha, I., Trushin, N., Freeman, W. M., & El-Bayoumy, K. (2009). Gene expression profiles in HPV-immortalized human cervical cells treated with the nicotine-derived carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Chem.-Biol. Interact., 177*, 173-180.

Prueitt, R. L., Yi, M., Hudson, R. S., Wallace, T. A., Howe, T. M., Yfantis, H. G., Lee, D. H., Stephens, R. M., Liu, C. G., Calin, G. A., Croce, C. M., & Ambs, S. (2008). Expression of microRNAs and protein-coding genes associated with perineural invasion in prostate cancer. *Prostate, 68*, 1152-1164.

ACCEPTED MANUSCRIPT

Puca, R., Nardinocchi, L., Bossi, G., Sacchi, A., Rechavi, G., Givol, D., & D'Orazi, G. (2009). Restoring wtp53 activity in HIPK2 depleted MCF7 cells by modulating metallothionein and zinc. *Exp. Cell Res., 315*, 67-75.

Pula, B., Tazbierski, T., Zamirska, A., Werynska, B., Bieniek, A., Szepietowski, J., Rys, J., Dziegiel, P., & Podhorska-Okolow, M. (2015). Metallothionein 3 Expression in Normal Skin and Malignant Skin Lesions. *Pathol. Oncol. Res., 21*, 187-193.

Raschke, M., Rowland, I. R., Magee, P. J., & Pool-Zobel, B. L. (2006). Genistein protects prostate cells against hydrogen peroxide-induced DNA damage and induces expression of genes involved in the defence against oxidative stress. *Carcinogenesis, 27*, 2322-2330.

Rodrigo, M. A. M., Zitka, O., Krizkova, S., Moulick, A., Adam, V., & Kizek, R. (2014). MALDI-TOF MS as evolving cancer diagnostic tool: A review. *J. Pharm. Biomed. Anal., 95*, 245-255.

Romero-Isart, N., & Vasak, M. (2002). Advances in the structure and chemistry of metallothioneins. *J. Inorg. Biochem., 88*, 388-396.

Rosen, M. D., Chan, I. H., & Privalsky, M. L. (2011). Mutant Thyroid Hormone Receptors (TRs) Isolated from Distinct Cancer Types Display Distinct Target Gene Specificities: A Unique Regulatory Repertoire Associated with Two Renal Clear Cell Carcinomas. *Mol. Endocrinol., 25*, 1311-1325.

Rossi, V., Bellastella, G., De Rosa, C., Abbondanza, C., Visconti, D., Maione, L., Chieffi, P., Della Ragione, F., Prezioso, D., De Bellis, A., Bellastella, A., & Sinisi, A. A. (2011). Raloxifene Induces Cell Death and Inhibits Proliferation Through Multiple Signaling Pathways in Prostate Cancer Cells Expressing Different Levels of Estrogen Receptor alpha and beta. *J. Cell. Physiol., 226*, 1334-1339.

Rudolf, E., & Cervinka, M. (2010). Zinc pyrithione induces cellular stress signaling and apoptosis in Hep-2 cervical tumor cells: the role of mitochondria and lysosomes. *Biometals, 23*, 339-354.

Ruttkay-Nedecky, B., Nejdl, L., Gumulec, J., Zitka, O., Masarik, M., Eckschlager, T., Stiborova, M., Adam, V., & Kizek, R. (2013). The Role of Metallothionein in Oxidative Stress. *Int. J. Mol. Sci., 14*, 6044-6066.

Ryu, H. H., Jung, S., Jung, T. Y., Moon, K. S., Kim, I. Y., Jeong, Y. I., Jin, S. G., Pei, J., Wen, M., & Jang, W. Y. (2012). Role of metallothionein 1E in the migration and invasion of human glioma cell lines. *Int. J. Oncol., 41*, 1305-1313.

Ryvolova, M., Krizkova, S., Adam, V., Beklova, M., Trnkova, L., Hubalek, J., & Kizek, R. (2011). Analytical Methods for Metallothionein Detection. *Curr. Anal. Chem., 7*, 243-261.

Sarafian, T., Habib, N., Mao, J. T., Tsu, I. H., Yamamoto, M. L., Hsu, E., Tashkin, D. P., & Roth, M. D. (2005). Gene expression changes in human small airway epithelial cells exposed to Delta(9)-tetrahydrocannabinol. *Toxicol. Lett., 158*, 95-107.

Sato, F., Tsuchiya, S., Meltzer, S. J., & Shimizu, K. (2011). MicroRNAs and epigenetics. *FEBS J., 278*, 1598-1609.

Savas, M. M., Shaw, C. F., & Petering, D. H. (1993). The oxidation of rabbit liver metallothionein-II by 5,5'-dithiobis(2-nitrobenzoic acid) and glutathione disulfide. *J. Inorg. Biochem., 52*, 235-249.

Scanlon, K. J., Kashanisabet, M., Tone, T., & Funato, T. (1991). Cisplatin resistance in human cancers. *Pharmacology & Therapeutics, 52*, 385-406.

Scaruffi, P., Morandi, F., Gallo, F., Stigliani, S., Parodi, S., Moretti, S., Bonassi, S., Fardin, P., Garaventa, A., Zanazzo, G., Pistoia, V., Tonini, G. P., & Corrias, M. V. (2012). Bone marrow of neuroblastoma patients shows downregulation of CXCL12 expression and presence of IFN signature. *Pediatr. Blood Cancer, 59*, 44-51.

ACCEPTED MANUSCRIPT

Scibetta, A. G., Santangelo, S., Coleman, J., Hall, D., Chaplin, T., Copier, J., Catchpole, S., Burchell, J., & Taylor-Papadimitriou, J. (2007). Functional analysis of the transcription repressor PLU-1/JARID1B. *Mol. Cell. Biol., 27*, 7220-7235.

Seibold, P., Hein, R., Schmezer, P., Hall, P., Liu, J. J., Dahmen, N., Flesch-Janys, D., Popanda, O., & Chang-Claude, J. (2011). Polymorphisms in oxidative stress-related genes and postmenopausal breast cancer risk. *Int. J. Cancer, 129*, 1467-1476.

Sens, M. A., Somji, S., Garrett, S. H., Beall, C. L., & Sens, D. A. (2001). Metallothionein isoform 3 overexpression is associated with breast cancers having a poor prognosis. *Am. J. Pathol., 159*, 21-26.

Shabb, J. B., Muhonen, W. W., & Mehus, A. A. (2017). Quantitation of Human Metallothionein Isoforms in Cells, Tissues, and Cerebrospinal Fluid by Mass Spectrometry. In A. K. Shukla (Ed.), *Proteomics in Biology, Pt B* (Vol. 586, pp. 413-431).

Sharma, S., Rais, A., Sandhu, R., Nel, W., & Ebadi, M. (2013). Clinical significance of metallothioneins in cell therapy and nanomedicine. *Int. J. Nanomed., 8*, 1477-1488.

Shin, C. H., Lee, M. G., Han, J., Jeong, S. I., Ryu, B. K., & Chi, S. G. (2017). Identification of XAF1-MT2A mutual antagonism as a molecular switch in cell-fate decisions under stressful conditions. *Proc. Natl. Acad. Sci. U. S. A., 114*, 5683-5688.

Schmidt, C. J., & Hamer, D. H. (1986). Cell specificity and an effect of ras on human metallothionein gene expression. *Proc. Natl. Acad. Sci. U. S. A., 83*, 3346-3350.

Singh, R. K., Albrecht, A. L., Somji, S., Sens, M. A., Sens, D. A., & Garrett, S. H. (2008). Alterations in metal toxicity and metal-induced metallothionein gene expression elicited by growth medium calcium concentration. *Cell Biol. Toxicol., 24*, 273-281.

Sirchia, R., Longo, A., & Luparello, C. (2008). Cadmium regulation of apoptotic and stress response genes in tumoral and immortalized epithelial cells of the human breast. *Biochimie, 90*, 1578-1590.

Sirchia, R., & Luparello, C. (2009). Short-term exposure to cadmium affects the expression of stress response and apoptosis-related genes in immortalized epithelial cells from the human breast. *Toxicol. Vitro, 23*, 943-949.

Skubitz, K. M., Francis, P., Skubitz, A. P. N., Luo, X. H., & Nilbert, M. (2012). Gene expression identifies heterogeneity of metastatic propensity in high-grade soft tissue sarcomas. *Cancer, 118*, 4235-4243.

Sliwinska-Mosson, M., Milnerowicz, H., Rabczynski, J., & Milnerowicz, S. (2009). Immunohistochemical localization of metallothionein and p53 protein in pancreatic serous cystadenomas. *Arch. Immunol. Ther. Exp., 57*, 295-301.

Slusser, A., Zheng, Y., Zhou, X. D., Somji, S., Sens, D. A., Sens, M. A., & Garrett, S. H. (2015). Metallothionein isoform 3 expression in human skin, related cancers and human skin derived cell cultures. *Toxicol. Lett., 232*, 141-148.

Smid-Koopman, E., Kuhne, L. C. M., Hanekamp, E. E., Gielen, S., De Ruiter, P. E., Grootegoed, J. A., Helmerhorst, T. J. M., Burger, C. W., Brinkmann, A. O., Huikeshoven, F. J., & Blok, L. J. (2005). Progesterone-induced inhibition of growth and differential regulation of gene expression in PRA- and/or PRB-expressing endometrial cancer cell lines. *J. Soc. Gynecol. Invest., 12*, 285-292.

Smith, D. J., Jaggi, M., Zhang, W., Galich, A., Du, C., Sterrett, S. P., Smith, L. M., & Balaji, K. C. (2006). Metallothioneins and resistance to cisplatin and radiation in prostate cancer. *Urology, 67*, 1341-1347.

Smith, E., Drew, P. A., Tian, Z. Q., De Young, N. J., Liu, J. F., Mayne, G. C., Ruszkiewicz, A. R., Watson, D. I., & Jamieson, G. G. (2005). Metallothionien 3 expression is frequently down-regulated in oesophageal squamous cell carcinoma by DNA methylation. *Mol. Cancer, 4*, 1-9.

ACCEPTED MANUSCRIPT

Sokolov, M. V., Panyutin, I. V., Panyutin, I. G., & Neumann, R. D. (2011). Dynamics of the transcriptome response of cultured human embryonic stem cells to ionizing radiation exposure. *Mutat. Res.-Fundam. Mol. Mech. Mutagen., 709-710*, 40-48.

Somji, S., Garrett, S., H., Zhou, X., D., Zheng, Y., Sens, D., A., & Sens, M., A. (2010). Absence of Metallothionein 3 Expression in Breast Cancer is a Rare, But Favorable Marker of Outcome that is Under Epigenetic Control. *Toxicol Environ. Chem., 92*, 1673-1695.

Somji, S., Garrett, S. H., Toni, C., Zhou, X. D., Zheng, Y., Ajjimaporn, A., Sens, M. A., & Sens, D. A. (2011). Differences in the epigenetic regulation of MT-3 gene expression between parental and Cd+2 or As+3 transformed human urothelial cells. *Cancer Cell Int., 11*, 1-14.

Somji, S., Sens, M. A., Lamm, D. L., Garrett, S. H., & Sens, D. A. (2001). Metallothionein isoform 1 and 2 gene expression in the human bladder: Evidence for upregulation of MT-1X mRNA in bladder cancer. *Cancer Detect. Prev., 25*, 62-75.

Soo, E. T. L., Ng, C. T., Yip, G. W. C., Koo, C. Y., Nga, M. E., Tan, P. H., & Bay, B. H. (2011). Differential Expression of Metallothionein in Gastrointestinal Stromal Tumors and Gastric Carcinomas. *Anat. Rec., 294*, 267-272.

Starska, K., Krzeslak, A., Forma, E., Olszewski, J., Lewy-Trenda, I., Osuch-Wojcikiewicz, E., & Brys, M. (2014). Genetic polymorphism of metallothionein 2A and risk of laryngeal cancer in a Polish population. *Med. Oncol., 31*, 1-10.

Starska, K., Krzeslak, A., Forma, E., Olszewski, J., Morawiec-Sztandera, A., Aleksandrowicz, P., Lewy-Trenda, I., & Brys, M. (2014). The-5 A/G single-nucleotide polymorphism in the core promoter region of MT2A and its effect on allele-specific gene expression and Cd, Zn and Cu levels in laryngeal cancer. *Toxicol. Appl. Pharmacol., 280*, 256-263.

Su, P. F., Lee, T. C., Lin, P. J., Lee, P. H., Jeng, Y. M., Chen, C. H., Liang, J. D., Chiou, L. L., Huang, G. T., & Lee, H. S. (2007). Differential DNA methylation associated with hepatitis B virus infection in hepatocellular carcinoma. *Int. J. Cancer, 121*, 1257-1264.

Subrungruang, I., Thawornkuno, C., Chawalitchewinkoon-Petmitr, P., Pairojkul, C., Wongkham, S., & Petmitr, S. (2013). Gene Expression Profiling of Intrahepatic Cholangiocarcinoma. *Asian Pac. J. Cancer Prev., 14*, 557-563.

Suganuma, K., Kubota, T., Saikawa, Y., Abe, S., Otani, Y., Furukawa, T., Kumai, K., Hasegawa, H., Watanabe, M., Kitajima, M., Nakayama, H., & Okabe, H. (2003). Possible chemoresistance-related genes for gastric cancer detected by cDNA microarray. *Cancer Sci., 94*, 355-359.

Sugita, K., Yamamoto, O., & Asahi, M. (2001). Immunohistochemical analysis of metallothionein expression in malignant melanoma in Japanese patients. *American Journal of Dermatopathology, 23*, 29-35.

Sun, X. F., Niu, X. H., Chen, R. C., He, W. Y., Chen, D., Kang, R., & Tang, D. L. (2016). Metallothionein-1G Facilitates Sorafenib Resistance Through Inhibition of Ferroptosis. *Hepatology, 64*, 488-500.

Sun, X. L., Jia, Y., Wei, Y. Y., Liu, S., & Yue, B. H. (2016). Gene expression profiling of NB4 cells following knockdown of nucleostemin using DNA microarrays. *Mol. Med. Rep., 14*, 175-183.

Szelachowska, J., Dziegiel, P., Tarkowski, R., Gomulkiewicz, A., Bebenek, M., Halon, A., Fortuna, K., Wojnar, A., Kornafel, J., & Matkowski, R. (2012). Therapeutic Radiation Induces Different Changes in Expression Profiles of Metallothionein (MT) mRNA, MT Protein, Ki 67 and Minichromosome Maintenance Protein 3 in Human Rectal Adenocarcinoma. *Anticancer Res., 32*, 5291-5297.

ACCEPTED MANUSCRIPT

Tahmasbpour, E., Ghanei, M., Qazvini, A., Vahedi, E., & Panahi, Y. (2016). Gene expression profile of oxidative stress and antioxidant defense in lung tissue of patients exposed to sulfur mustard. *Mutat. Res. Genet. Toxicol. Environ. Mutagen., 800*, 12-21.

Tai, S. K., Tan, O. J. K., Chow, V. T. K., Jin, R. X., Jones, J. L., Tan, P. H., Jayasurya, A., & Bay, B. H. (2003). Differential expression of metallothionein 1 and 2 lsoforms in breast cancer lines with different invasive potential - Identification of a novel nonsilent metallothionein-1H mutant variant. *Am. J. Pathol., 163*, 2009-2019.

Takahashi, M., Rhodes, D. R., Furge, K. A., Kanayamat, H., Kagawa, S., Haab, B. B., & Teh, B. T. (2001). Gene expression profiling of clear cell renal cell carcinoma: Gene identification and prognostic classification. *Proc. Natl. Acad. Sci. U. S. A., 98*, 9754-9759.

Takahashi, S. (2015). Positive and negative regulators of the metallothionein gene (Review). *Mol. Med. Rep., 12*, 795-799.

Takata, A., Otsuka, M., Yoshikawa, T., Kishikawa, T., Hikiba, Y., Obi, S., Goto, T., Kang, Y. J., Maeda, S., Yoshida, H., Omata, M., Asahara, H., & Koike, K. (2013). MicroRNA-140 acts as a liver tumor suppressor by controlling NF-kappa B activity by directly targeting DNA methyltransferase 1 (Dnmt1) expression. *Hepatology, 57*, 162-170.

Takeda, A., Hisada, H., Okada, S., Mata, J. E., Ebadi, M., & Iversen, P. L. (1997). Tumor cell growth is inhibited by suppressing metallothionein-I synthesis. *Cancer Lett., 116*, 145-149.

Tan, H. T., Tan, S., Lin, Q. S., Lim, T. K., Hew, C. L., & Chung, M. C. M. (2008). Quantitative and temporal proteome analysis of butyrate-treated colorectal cancer cells. *Mol. Cell. Proteomics, 7*, 1174-1185.

Tan, O. J. K., Bay, B. H., & Chow, V. T. K. (2005). Differential expression of metallothionein isoforms in nasopharyneal cancer and inhibition of cell growth by antisense down-regulation of metallothionein-2A. *Oncol. Rep., 13*, 127-131.

Tao, L., Forester, S. C., & Lambert, J. D. (2014). The role of the mitochondrial oxidative stress in the cytotoxic effects of the green tea catechin, (-)-epigallocatechin-3-gallate, in oral cells. *Mol. Nutr. Food Res., 58*, 665-676.

Tao, X., Zheng, J. M., Xu, A. M., Chen, X. F., & Zhang, S. H. (2007). Downregulated expression of metallothionein and its clinicopathological significance in hepatocellular carcinoma. *Hepatol. Res., 37*, 820-827.

Tao, Y. F., Xu, L. X., Lu, J., Cao, L., Li, Z. H., Hu, S. Y., Wang, N. N., Du, X. J., Sun, L. C., Zhao, W. L., Xiao, P. F., Fang, F., Li, Y. H., Li, G., Zhao, H., Li, Y. P., Xu, Y. Y., Ni, J., Wang, J., Feng, X., & Pan, J. (2014). Metallothionein III (MT3) is a putative tumor suppressor gene that is frequently inactivated in pediatric acute myeloid leukemia by promoter hypermethylation. *J. Transl. Med., 12*, 1-14.

Tarapore, P., Shu, Y., Guo, P. X., & Ho, S. M. (2011). Application of Phi29 Motor pRNA for Targeted Therapeutic Delivery of siRNA Silencing Metallothionein-IIA and Survivin in Ovarian Cancers. *Mol. Ther., 19*, 386-394.

Telang, U., Braeau, D. A., & Morris, M. E. (2009). Comparison of the Effects of Phenethyl Isothiocyanate and Sulforaphane on Gene Expression in Breast Cancer and Normal Mammary Epithelial Cells. *Exp. Biol. Med., 234*, 287-295.

Teschendorff, A. E., Menon, U., Gentry-Maharaj, A., Ramus, S. J., Weisenberger, D. J., Shen, H., Campan, M., Noushmehr, H., Bell, C. G., Maxwell, A. P., Savage, D. A., Mueller-Holzner, E., Marth, C., Kocjan, G., Gayther, S. A., Jones, A., Beck, S., Wagner, W., Laird, P. W., Jacobs, I. J., & Widschwendter, M. (2010). Age-dependent DNA methylation of genes that are suppressed in stem cells is a hallmark of cancer. *Genome Res., 20*, 440-446.

ACCEPTED MANUSCRIPT

Theocharis, S. E., Margeli, A. P., Klijanienko, J. T., & Kouraklis, G. P. (2004). Metallothionein expression in human neoplasia. *Histopathology, 45*, 103-118.

Thirumoorthy, N., Sunder, A. S., Kumar, K. T. M., Kumar, M. S., Ganesh, G. N. K., & Chatterjee, M. (2011). A Review of Metallothionein Isoforms and their Role in Pathophysiology. *World J. Surg. Oncol., 9*, 1-7.

Thompson, C. A., & Burcham, P. C. (2008). Genome-Wide Transcriptional Responses to Acrolein. *Chem. Res. Toxicol., 21*, 2245-2256.

Tchounwou, P. B., Yedjou, C. G., Foxx, D. N., Ishaque, A. B., & Shen, E. (2004). Lead-induced cytotoxicity and transcriptional activation of stress genes in human liver carcinoma (HepG(2)) cells. *Mol. Cell. Biochem., 255*, 161-170.

Tian, Z. Q., Xu, Y. Z., Zhang, Y. F., Ma, G. F., He, M., & Wang, G. Y. (2013). Effects of metallothionein-3 and metallothionein-1E gene transfection on proliferation, cell cycle, and apoptosis of esophageal cancer cells. *Genet. Mol. Res., 12*, 4595-4603.

Tiwari, R., Pandey, S. K., Goel, S., Bhatia, V., Shukla, S., Jing, X., Dhanasekaran, S. M., & Ateeq, B. (2015). SPINK1 promotes colorectal cancer progression by downregulating Metallothioneins expression. *Oncogenesis, 4*, 1-12.

Tripathi, M. K., Misra, S., & Chaudhuri, G. (2005). Negative regulation of the expressions of cytokeratins 8 and 19 by SLUG repressor protein in human breast cells. *Biochem. Biophys. Res. Commun., 329*, 508-515.

Tse, K. Y., Liu, V. W. S., Chan, D. W., Chiu, P. M., Tam, K. F., Chan, K. K. L., Liao, X. Y., Cheung, A. N. Y., & Ngan, H. Y. S. (2009). Epigenetic Alteration of the Metallothionein 1E Gene in Human Endometrial Carcinomas. *Tumor Biol., 30*, 93-99.

Tsou, J. A., Galler, J. S., Wali, A., Ye, W., Siegmund, K. D., Groshen, S., Laird, P. W., Turla, S., Koss, M. N., Pass, H. I., & Laird-Offringa, I. A. (2007). DNA methylation profile of 28 potential marker loci in malignant mesothelioma. *Lung Cancer, 58*, 220-230.

Tuzel, E., Kirkali, Z., Yorukoglu, K., Mungan, M. U., & Sade, M. (2001). Metallothionein expression in renal cell carcinoma: subcellular localization and prognostic significance. *J. Urol., 165*, 1710-1713.

Volm, M. (1998). Multidrug resistance and its reversal. *Anticancer Res., 18*, 2905-2917.

Waddington, C. H. (1942). *The Epigenotype* (Vol. 1): Endeavour.

Wang, R. Y., Sens, D. A., Albrecht, A., Garrett, S., Somji, S., Sens, M. A., & Lu, X. N. (2007). Simple method for identification of metallothionein isoforms in cultured human prostate cells by MALDI-TOF/TOF mass spectrometry. *Anal. Chem., 79*, 4433-4441.

Wei, H., Desouki, M. M., Lin, S., Xiao, D., Franklin, R. B., & Feng, P. (2008). Differential expression of metallothioneins (MTs) 1, 2, and 3 in response to zinc treatment in human prostate normal and malignant cells and tissues. *Mol. Cancer, 7*, 1-11.

Weinlich, G. (2009). Metallothionein-overexpression as a prognostic marker in melanoma. *G. Ital. Dermatol. Venereol., 144*, 27-38.

Werynska, B., Pula, B., Muszczynska-Bernhard, B., Gomulkiewicz, A., Jethon, A., Podhorska-Okolow, M., Jankowska, R., & Dziegiel, P. (2013). Expression of Metallothionein-III in Patients with Non-small Cell Lung Cancer. *Anticancer Res., 33*, 965-974.

Werynska, B., Pula, B., Muszczynska-Bernhard, B., Gomulkiewicz, A., Piotrowska, A., Prus, R., Podhorska-Okolow, M., Jankowska, R., & Dziegiel, P. (2013). Metallothionein IF and 2A overexpression predicts poor outcome of non-small cell lung cancer patients. *Exp. Mol. Pathol., 94*, 301-308.

Wierzowiecka, B., Gomulkiewicz, A., Cwynar-Zajac, L., Olbromski, M., Grzegrzolka, J., Kobierzycki, C., Podhorska-Okolow, M., & Dziegiel, P. (2016). Expression of

ACCEPTED MANUSCRIPT

Metallothionein and Vascular Endothelial Growth Factor Isoforms in Breast Cancer Cells. *In Vivo, 30*, 271-278.

Wong, R. H., Huang, C. H., Yeh, C. B., Lee, H. S., Chien, M. H., & Yang, S. F. (2013). Effects of Metallothionein-1 Genetic Polymorphism and Cigarette Smoking on the Development of Hepatocellular Carcinoma. *Ann. Surg. Oncol., 20*, 2088-2095.

Woolston, C. M., Deen, S., Al-Attar, A., Shehata, M., Chan, S. Y., & Martin, S. G. (2010). Redox protein expression predicts progression-free and overall survival in ovarian cancer patients treated with platinum-based chemotherapy. *Free Radic. Biol. Med., 49*, 1263-1272.

Worthington, J., Bertani, M., Chan, H. L., Gerrits, B., & Timms, J. F. (2010). Transcriptional profiling of ErbB signalling in mammary luminal epithelial cells - interplay of ErbB and IGF1 signalling through IGFBP3 regulation. *BMC Cancer, 10*, 1-22.

Wu, Y., Siadaty, M. S., Berens, M. E., Hampton, G. M., & Theodorescu, D. (2008). Overlapping gene expression profiles of cell migration and tumor invasion in human bladder cancer identify metallothionein 1E and nicotinamide N-methyltransferase as novel regulators of cell migration. *Oncogene, 27*, 6679-6689.

Wulfing, C., van Ahlen, H., Eltze, E., Piechota, H., Hertle, L., & Schmid, K. W. (2007). Metallothionein in bladder cancer: correlation of overexpression with poor outcome after chemotherapy. *World J. Urol., 25*, 199-205.

Yamasaki, M., Nomura, T., Sato, F., & Mimata, H. (2007). Metallothionein is up-regulated under hypoxia and promotes the survival of human prostate cancer cells. *Oncol. Rep., 18*, 1145-1153.

Yan, D. W., Fan, J. W., Yu, Z. H., Li, M. X., Wen, Y. G., Li, D. W., Zhou, C. Z., Wang, X. L., Wang, Q., Tang, H. M., & Peng, Z. H. (2012). Downregulation of Metallothionein 1F, a putative oncosuppressor, by loss of heterozygosity in colon cancer tissue. *Biochim. Biophys. Acta-Mol. Basis Dis., 1822*, 918-926.

Yang, J., Zhang, Y. B., Liu, P., Yan, H. L., Ma, J. C., & Da, M. X. (2017). Decreased expression of long noncoding RNA MT1JP may be a novel diagnostic and predictive biomarker in gastric cancer. *Int. J. Clin. Exp. Pathol., 10*, 432-438.

Yang, X., Song, J. H., Cheng, Y. L., Wu, W. J., Bhagat, T., Yu, Y. T., Abraham, J. M., Ibrahim, S., Ravich, W., Roland, B. C., Khashab, M., Singh, V. K., Shin, E. J., Verma, A. K., Meltzer, S. J., & Mori, Y. (2014). Long non-coding RNA HNF1A-AS1 regulates proliferation and migration in oesophageal adenocarcinoma cells. *Gut, 63*, 881-890.

Yang, Y. Y., Woo, E. S., Reese, C. E., Bahnson, R. R., Saijo, N., & Lazo, J. S. (1994). Human metallothionein isoform gene expression in cisplatin-sensitive and resistant cells. *Mol. Pharmacol., 45*, 453-460.

Yap, X. L., Tan, H. Y., Huang, J. X., Lai, Y. Y., Yip, G. W. C., Tan, P. H., & Bay, B. H. (2009). Over-expression of metallothionein predicts chemoresistance in breast cancer. *J. Pathol., 217*, 563-570.

Yin, H., Smith, M., & Glass, J. (2005). Stable expression of C/EBP alpha in prostate cancer cells down-regulates metallothionein and increases zinc-induced toxicity. *Prostate, 62*, 209-216.

Yu, W. J., Qiao, Y. X., Tang, X., Ma, L. F., Wang, Y. L., Zhang, X., Weng, W. H., Pan, Q. H., Yu, Y. C., Sun, F. Y., & Wang, J. Y. (2014). Tumor suppressor long non-coding RNA, MT1DP is negatively regulated by YAP and Runx2 to inhibit FoxA1 in liver cancer cells. *Cell. Signal., 26*, 2961-2968.

Zalewska, M., Trefon, J., & Milnerowicz, H. (2014). The role of metallothionein interactions with other proteins. *Proteomics, 14*, 1343-1356.

ACCEPTED MANUSCRIPT

Zamirska, A., Matusiak, L., Dziegiel, P., Szybejko-Machaj, G., & Szepietowski, J. C. (2012). Expression of metallothioneins in cutaneous squamous cell carcinoma and actinic keratosis. *Pathol. Oncol. Res., 18*, 849-855.

Zavras, A. I., Yoon, A. J., Chen, M. K., Lin, C. W., & Yang, S. F. (2011). Metallothionein-1 Genotypes in the Risk of Oral Squamous Cell Carcinoma. *Ann. Surg. Oncol., 18*, 1478-1483.

Zeisig, R., Koklic, T., Wiesner, B., Fichtner, I., & Sentjurc, M. (2007). Increase in fluidity in the membrane of MT3 breast cancer cells correlates with enhanced cell adhesion in vitro and increased lung metastasis in NOD/SCID mice. *Archives of Biochemistry and Biophysics, 459*, 98-106.

Zhang, W. C., Chin, T. M., Yang, H., Nga, M. E., Lunny, D. P., Lim, E. K. H., Sun, L. L., Pang, Y. H., Leow, Y. N., Malusay, S. R. Y., Lim, P. X. H., Lee, J. Z., Tan, B. J. W., Shyh-Chang, N., Lim, E. H., Lim, W. T., Tan, D. S. W., Tan, E. H., Tai, B. C., Soo, R. A., Tam, W. L., & Lim, B. (2016). Tumour-initiating cell-specific miR-1246 and miR-1290 expression converge to promote non-small cell lung cancer progression. *Nat. Commun., 7*, 1-16.

Zheng, G. P., Zhou, M., Ou, X. R., Peng, B., Yu, Y. H., Kong, F. R., Ouyang, Y. M., & He, Z. M. (2010). Identification of carbonic anhydrase 9 as a contributor to pingyangmycin-induced drug resistance in human tongue cancer cells. *FEBS J., 277*, 4506-4518.

Zheng, X. H., Watts, G. S., Vaught, S., & Gandolfi, A. J. (2003). Low-level arsenite induced gene expression in HEK293 cells. *Toxicology, 187*, 39-48.

Zheng, Y. L., Jiang, L. H., Hu, Y. X., Xiao, C., Xu, N., Zhou, J. Y., & Zhou, X. H. (2017). Metallothionein 1H (MT1H) functions as a tumor suppressor in hepatocellular carcinoma through regulating Wnt/beta-catenin signaling pathway. *BMC Cancer, 17*, 1-11.

Zhong, S., Fields, C. R., Su, N., Pan, Y. X., & Robertson, K. D. (2007). Pharmacologic inhibition of epigenetic modifications, coupled with gene expression profiling, reveals novel targets of aberrant DNA methylation and histone deacetylation in lung cancer. *Oncogene, 26*, 2621-2634.

Zhou, X. D., Sens, M. A., Garrett, S. H., Somji, S., Park, S., Gurel, V., & Sens, D. A. (2006). Enhanced expression of metallothionein isoform 3 protein in tumor heterotransplants derived from As+3- and Cd+2-transformed human urothelial cells. *Toxicol. Sci., 93*, 322-330.