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Regulation of transcription in cancer

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TABLE OF CONTENTS

1. ABSTRACT

The purpose of this article is to provide an overview on the regulation of transcription in cancer cells. We describe here standard mechanisms of transcription in eukaryotic cells, an influence of common promoter polymorphisms contributing to malignant progression and DNA methylation as significant aspect of gene regulation. We also described transcription factors mechanism of action, and how their alteration can result in cancer.

2. INTRODUCTION

Regulatory sequences, mainly promoter and its core region are involved in the process of gene regulation and play an indispensable, central role in gene expression. Other elements of gene regulation are transcription factors that affect the expression of structural genes and DNA methylation which is involved in regulation of many cellular processes. Alterations in any of these three aspects of gene control can result in cancer. In this review we

summarized the recent advances in understanding the regulation of gene expression on the level of promoter, its epigenetic modification and transcription factors influence.

3. TRANSCRIPTIONAL MACHINERY

3.1. Gene promoter

Cellular homeostasis necessary for normal growth and development is an outcome of precise control exerted by a complex transcriptional machinery. In eukaryotes, the central part of this system is RNA polymerase type II (Pol II), which drives the transcription of protein-encoding genes. Pol II and its accompanying proteins recognize and bind to specific motifs in DNA, which include promoters, enhancers, silencers and boundary (insulator) elements.

The promoter is central to transcription initiation and spans approx. from -250 to +250 bp relative to the transcription start site (TSS). The integral part of each promoter is a so-called core promoter (discussed in detail in subsequent sections), which encompasses the TSS and directs the initiation of transcription by recruiting General Transcription Factors (GTFs). The region immediately adjacent to the core promoter, termed promoter-proximal region or extended promoter, features multiple binding sites for specific transcription factors that modulate the basal transcriptional activity of Pol II-GTF complex. The transcription initiation is further regulated from far more distal positions by enhancers and silencers, located even tens of kbp upstream or downstream from TSS. Additional *cis*-acting DNA components include boundary (insulator) elements, believed to play a role in abolishing the reciprocal influence of neighbouring transcriptional units (1, 2).

With regard to the mode of transcription initiation the core promoters can be divided into two major types: focused and dispersed. A typical focused core promoter is characterized by a one or a few discrete transcription initiation sites within a short nucleotide region, whereas a dispersed promoter does not exhibit a single, well-established transcription start site, but rather features multiple weak initiation sites over a stretch of 50-150 nucleotides (3).

3.2. Core promoter motifs

The core promoter is the minimal portion of the promoter required to properly initiate transcription. It is located approximately 40 bp of the start site. It includes transcription start site for general transcription factors and RNA polymerase I (transcribes genes encoding ribosomal RNA), polymerase II (transcribes genes encoding messenger RNA and some small nuclear RNAs) and polymerase III (transcribes genes encoding tRNAs). The core promoter is found in all protein-coding genes (4). Various sequence motifs, such as TATA box, which is the most ancient promoter motif, initiator (Inr), the fragment containing the transcription start site, transcription factor IIB recognition element (BRE), motif ten element (MTE), downstream core promoter element (DPE) and downstream core element (DCE) are common components of the focused core promoters. The classical TATA box describes a nucleotide octamer with a defined consensus sequence of TATAWAAR (W=A/T, R=A/G). This most ancient of the promoter motifs is usually located ca. 30 bp upstream from the TSS and it serves to provide a docking site for TATA box-binding protein (TBP). In addition, the TATA box is also occasionally flanked by a sequence termed Transcription Factor IIB (TFIIB) recognition element, that can reside on either upstream or downstream side of the TATA box and – according to the promoter context – exerts different modulatory effects.

Another commonly occurring core promoter element is Inr, which contains the transcription start site and participates in assembly of TFIID complex by providing a binding platform for TBP-associated factors 1 and 2 (TAF1 and TAF2). In contrast, other TFIID subunits, TAF6 and TAF9, assemble close to DPE. This sequence motif is located from +28 to +33 bp relative to the TSS and cooperates with Inr in steering transcription initiation. Inr, TATA box and DPE are also known to act together with MTE, which is located slightly upstream from DPE (Figure 1).

Furthermore, TATA box occasionally cooperates with yet another motif, DCE, entailing three short DNA domains. Notably, all of the above mentioned motifs appear only in a small fraction of the identified promoters. Although some of the elements tend to cluster together and act cooperatively, such as TATA box and BRE or DPE and Inr, some promoters feature only a single distinct motif and a number of core promoters appear to be completely devoid of any functional units characterized so far (4).

3.3. Relative frequency of different promoter types in human genome

A recent study by Yang *et al.* established the relative frequency of different core promoter motifs in human genome (5). The canonical TATA box and its various derivatives appear in only as little as 24% of human genes. In contrast to the remaining 76% of promoters that display high GC fraction and multiple Sp1 binding sites, the TATA-containing promoters are usually focused and rich in AT pairs.

The Inr element is slightly more widespread than TATA box. Despite the fact that Inr often coincides with TATA box, it also appears independently in many transcriptional units.

The 46% of human core promoters are devoid of these two classical motifs, Inr and TATA box, and are based solely on CpG islands to initiate the transcription (5). The GC-rich regions often span from 0.5 to as much as 2 kbp and feature numerous consensus binding sites for Sp1, Elk-1 and other factors. Due to their dispersed nature it is plausible that GC-rich promoters contain a range of weak transcription start sites and the abundance of CpG motifs allows for epigenetic silencing by the control of methylation status (1).

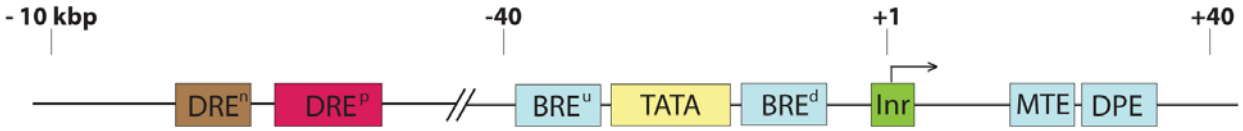

Figure 1. Various components of the focused core promoters. DRE p/n - distal regulatory element positive/negative, BRE u/d - transcription factor IIB recognition element upstream/downstream, TATA - the most ancient promoter motif, Inr- initiator containing the transcription start site, MTE - motif ten element, DPE - downstream core promoter element.

3.4. Relationship between promoter structure and function

Interestingly, the types of core promoter motifs seem to correspond to the way in which their respective genes are expressed. This correlation refers to the duration of transcription, intracellular location of coded proteins and the pattern of tissue expression. For example, TATA-containing promoters are subjected to a strict transcriptional control and tend to be induced only under specific conditions, quite unlike the TATA-less promoters, which typically drive the expression of 'housekeeping' genes (5). What is more, the core promoter elements supposedly correlate with the compartmentalization of proteins. TATA-box motif in the promoter sequence is often associated with extracellular destination of proteins, CpG islands with nuclear or mitochondrial address and promoters without TATA box or CpG islands with membrane localization (6). Furthermore, focused promoters are strongly overrepresented in genes coding for the tissue-specific transcripts (6, 7).

Another intriguing issue is the existence of 'bidirectional' promoters, which are primarily located within the CpG islands and regulate the expression of about 10% of the human genes. The genes controlled by this type of promoters are arranged in head-to-head manner and their TSSs lie in relative close proximity (less than 1000 bp apart). Apart from some rare cases, in which the expression of gene pairs is reciprocally negatively regulated, the majority of bidirectional promoters positively correlate with the transcription of their respective genes. This phenomenon may play an important role for genes that require transcription synchronized in time or on the same level (7, 8).

The genes possessing two or more functional alternate promoters are even more common (20%) than 'bidirectional' promoters. The possibility of differential transcription initiation provides yet another mechanism of gene expression control. Specifically, the selection of a particular promoter from the bigger assortment is often determined by tissue type and occasionally results in production of altered protein products (7, 9).

3.5. Core promoter elements as docking sites for transcriptional machinery

The wide array of motifs present in core promoters serves to recruit GTFs to the transcription start site and promote the assembly of a preinitiation complex (PIC). According to two different hypotheses, the complex formation takes place in either a step-wise manner or by complete holoenzyme binding. In the classical model TATA box is initially recognized by TBP, which together with TAFs forms TFIID complex. Further recruitment of TFIIA and TFIIB precedes the docking of Pol II-TFIIF complex. However, the formation of an open complex requires yet another additional factors: TFIIE and TFIIH, which together cooperate in opening and stabilizing the transcription bubble. TFIIH – apart from helicase activity – is also endowed with kinase properties and therefore it can participate in control of Pol II phosphorylation status. More precisely, it is the C-terminal domain (CTD) of Pol II that undergoes covalent modifications and, depending on its phosphorylation state, the CTD interacts with diverse proteins responsible for transcription initiation, elongation and termination (10, 11). One of the factors binding to hypophosphorylated CTD is Mediator, a large complex consisting of about 20 subunits also referred to as coactivating comlex. Although much less recognized than the pool of GTFs, Mediator is essential for processing and integrating regulatory signals for transcription by interacting directly with both Pol II and activator proteins coupled to enhancer elements (12) (Figure 2).

Finally, due to the concerted activity of GTFs, Mediator and Pol II the unstable open complex is formed and the synthesis of first RNA bonds takes place. After several rounds resulting in abortive products, the polymerase finally leaves the promoter and accompanied only by a set of specific elongation factors proceeds to form a full-length product. Some of the GTFs remain associated with the promoter to initiate next transcription round (11).

3.6. Other transcription controlling sites

Eukaryotic promoters can have transcription regulatory sites hundreds or thousands of base pairs from the core promoter, upstream, downstream, or even within the gene they control. Although GTFs and Pol II alone are able to promote transcription, this process is usually very unproductive. Therefore, to achieve an efficient transcription a multitude of accompanying transcription factors is involved in the process. Apart from basic transcriptional machinery a wide range of different transcription factors is involved: either tissue specific or expressed ubiquitously, present constitutively or only transiently under certain conditions, imposing negative or positive modulatory effects. The binding of transcription factors to each other probably draws the DNA of the promoter into a loop, which allows for placement of regulatory sequences far from the actual site of transcription. Transcription factors binding to these fragments are called enhancers or silencers (13) (see Figure 2). The sequence from -40 to -350 relative to the TSS is usually associated with enhancement in promoter activity,

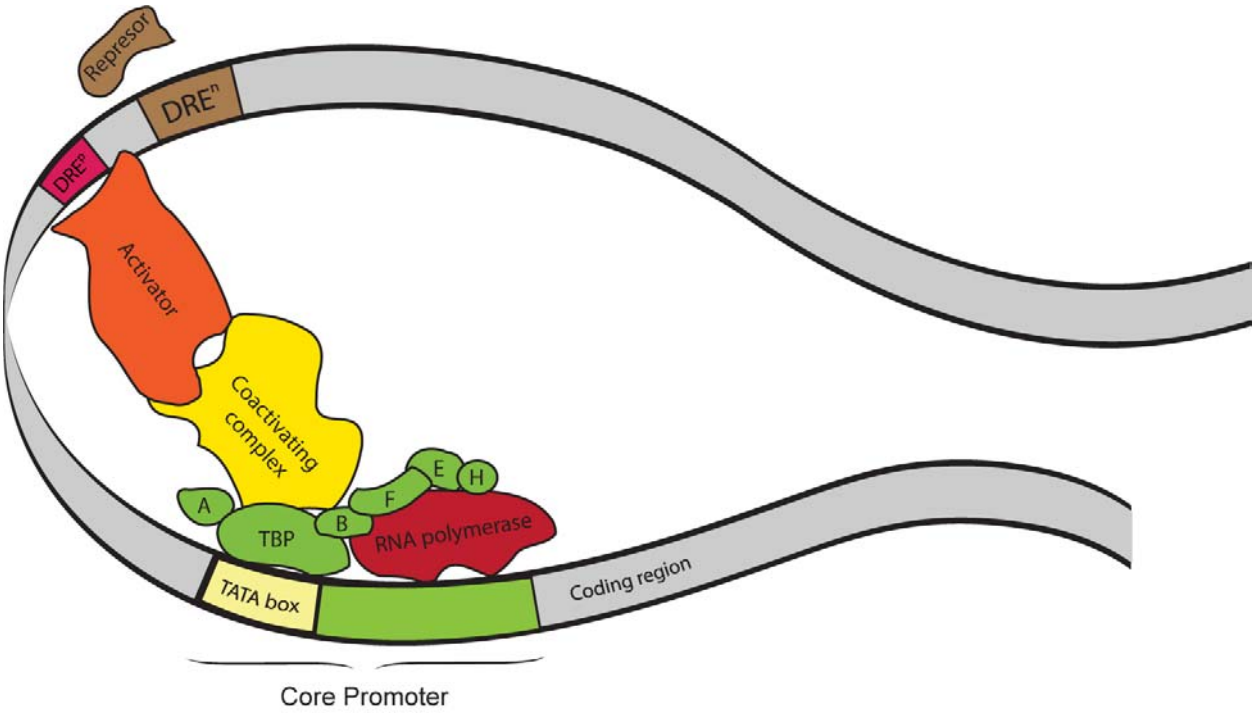

Figure 2. Assembly of a preinitiation complex. TBP – TATA box-binding protein, A, B, F, E, H –transcription factors involved in assembly of basic transcriptional complex, DRE - DRE p/n - distal regulatory element positive/negative.

while the region from -350 to -1000 tends to negatively affect transcription initiation (9). Enhancer elements impose further positive effect on gene expression due to recruiting diverse factors that influence the transcription levels by protein-protein interactions, covalent modification of proteins, control of chromatin structure or nucleosome remodelling (1).

4. POLYMORPHISMS IN THE PROMOTER REGION CONTRIBUTING TO MALIGNANT PROGRESSION

The existence of common promoter polymorphisms such as single nucleotide polymorphisms (SNPs), short tandem repeats (STRs) or variable number of tandem repeats (VNTRs) can alter the gene expression profile of an individual. Such genetic variations commonly present in the population are one of the reasons why some people are at higher risk of developing certain cancers, suffer from faster progression to malignant stage and differentially respond to the applied therapy. The cumulative knowledge of low-penetrance genetic factors may be therefore useful for diagnostic testing and introducing appropriate treatment.

Several examples listed below illustrate how polymorphisms in the promoter region contribute to the different stages of malignant progression.

4.1. Mismatch repair

In the mismatch repair system proteins such as MLH1, MSH2, MSH6 and PMS*2* guard the accurate replication of the genome. The inactivation of mismatch repair system mechanisms leads to microsatellite instability. Consistent with this, *MLH1* -93G>A promoter polymorphism was recently reported to be linked with increased risk of developing microsatellite unstable colorectal cancer. According to the study, the A allele contributes to the reduced levels of protein and subsequent microsatellite instability phenotype due to the higher susceptibility to promoter methylation (13, 14). The very same genotype also plays a role in the increased risk of invasive ovarian cancer (15) and endometrial cancer (16).

Extensive studies are aimed to test the links between the detected polymorphisms and susceptibility to malignant diseases. For instance, putative role of a 13bp duplication in promoter region of Fanconi anemia, complementation group A (*FANCA*),a factor involved in response to DNA damage, was suggested as a low-penetrance (unfrequent in population) protective factor for ovarian cancer (17).

4.2. Proliferation control and apoptosis

The most well-known tumor suppressor gene is *p53*, a protein that upon activation by factors such as DNA damage, oxidative stress or heat shock, imposes the cell cycle arrest and apoptosis. One of its effector genes is murine double minute (*Mdm2*) that in turn inhibits p53 activity, creating a negative feedback loop. Common 309T>G SNP in *Mdm2* promoter was reported to influence cancer progression due to enhanced Sp1 (specificity protein 1, described more in “Transcriptional control” chapter in this article) binding, higher protein expression and subsequent attenuating of p53 pathway (18). In succeeding

studies on various malignancies, such as neuroblastoma (19), pancreatic carcinoma (20) or renal cell carcinoma (21), the G allele of *Mdm2* promoter was repeatedly associated with higher risk, worse prognosis and poorer survival. However, the very same polymorphism in a combined study by Wilkening *et al.* was determined to lack or have little impact on the risk of common cancers (no effect – breast or colorectal cancers, little effect – lung cancer), but its influence on tumor onset time and prognosis was not excluded (22).

The prognostic role of a common promoter P2 B-cell lymphoma (*Bcl-2*) polymorphism is surprisingly disparate with respect to different cancer types. The main role of this regulatory factor is to protect cells from apoptosis by inhibiting cell cycle in G_0 phase and thus acting in an anti-proliferative manner. Interestingly, depending on the cancer type, *Bcl-2* can play a role either es tumor oncogene or suppressor (23). This dual role in cancer progression is also reflected by the -938C>A polymorphism: the A allele – associated with higher Bcl-2 levels – is a favourable prognostic and survival factor in breast cancer node-negative patients (23), whereas in B-cell chronic lymphocytic leukemia it is linked with poorer prognosis and outcome (24).

4.3. Modified cell adhesion

The key element necessary for metastasis formation is the acquisition of the migratory properties by the tumor cells. The first barrier that has to be overcome in cell migration is the detachment from the primary location. To achieve this aim the cell switches the pattern of recognition molecules expressed on the surface. One of the proteins that often undergoes downregulation in epithelial cancers is E-cadherin. Due to maintenance of intercellular contacts and preserving cell differentiation status it functions as a suppressor of tumor invasion and metastasis (25). Therefore, the early report stating that -160C>A polymorphism affects *E-cadherin* expression by differential binding of transcription factors received much attention (26). The SNP was extensively studied with regard to different cancer types, e.g. primary non-small cell lung cancer (27). Additionally, the very recent meta-analysis confirmed that A allele is indeed associated with slightly elevated risk of several invasive tumors, including gastric, lung, urothelial and prostate cancer, thus validating the -160C>A SNP as a low-penetrance susceptibility factor in tumor progression (28). Other polymorphisms of *E-cadherin* promoter, such as -347G>GA insertion decreasing the transcription by approximately 10-fold *in vitro* (29), are also investigated in context of enhanced predisposition to various types of epithelial cancers.

4.4. Matrix degradation

In order to enter the bloodstream and later to form metastases in other tissues, the invading cell has to find its way towards the vessel. Consequently, it produces several matrix degrading proteins to disintegrate the extracellular environment in a polarized fashion. Such matrix-degrading proteins include matrix metalloproteinase-1 (MMP-1), also known as collagenase. Since the discovery of a common -1607G>GG polymorphism in the promoter region of *MMP-1*, a number of attempts were dedicated to elucidate the role of this SNP in various cancers. The insertion of the additional guanine was reported to create a novel Ets-1 binding site and enhance gene transcription (30). Thus, as a result of augmented *MMP-1* expression, the GG genotype was hypothesised to increase the risk of invasive cancer and diminish patient survival. This attractive assumption was tested in several studies, which produced contradictory results. For instance, the earlier research papers on colorectal cancer (CRC) invasiveness suggested the role of GG allele as a factor in increased tumor growth and metastasis (31), while a more recent analysis associated this genotype with more favourable prognosis in CRC (32). In addition, some studies showed weak correlation between the polymorphism and increased cancer risk (e.g. lung cancer study (33)), whereas no link was found in other attempts (e.g. cervical cancer study in Korean population (34)). The report that may shed some light on the role of *MMP-1* promoter polymorphism outlines the impact of the whole haplotype in *MMP-1* expression. According to the authors, the cumulative effect of several promoter polymorphisms may lead to expression variations and thus the haplotype rather than single SNPs should be related to pathogenic states (35).

4.5. Promoter polymorphisms and cancer

With the growing number of reports and emerging scientific evidence the full list of genetic polymorphisms influencing cancerous process is far from complete. Since 2004 state of knowledge progressed significantly, especially with the completion of several major world-wide projects such as ENCODE (Encyclopedia of DNA elements) pilot project analysing functional elements in 1% human genome or Phase II of International HapMap Project, which characterized over 3.1 million human SNPs (36, 37, 38).

SNPs and other variations in promoter regions contribute at least partially to the organism response to external as well as internal factors. The individual genetic setting influences the risk of developing tumors by disparate activity of genome repair system, xenobiotic metabolism and endocrine mechanisms. Moreover, small variations in gene expression patterns can also tip the balance towards faster cancer progression, more invasive phenotype and metastases formation. Different genetic contexts also influence drug metabolism, response to therapy and overall cytotoxicity of the treatment.

In experiments performed in our laboratory we look for mutations in MET promoter, which is crucial in oncogenic pathways and metastatic behaviour of tumor cells (39). Promoter sequencing revealed some dispersed and random alterations in rhabdomyosarcoma samples. However, the most common substitutions are -304C>A and +206C>G (Figure 3).

Mutation -304C>A disrupts putative binding sites for Sp1 and AP-1/AP-2, but introduces Gata-1/Gata-2 and E47/E12 binding sites. Mutation +206C>G disrupts Sp1 and GCF putative binding sites. The identified mutations are to be

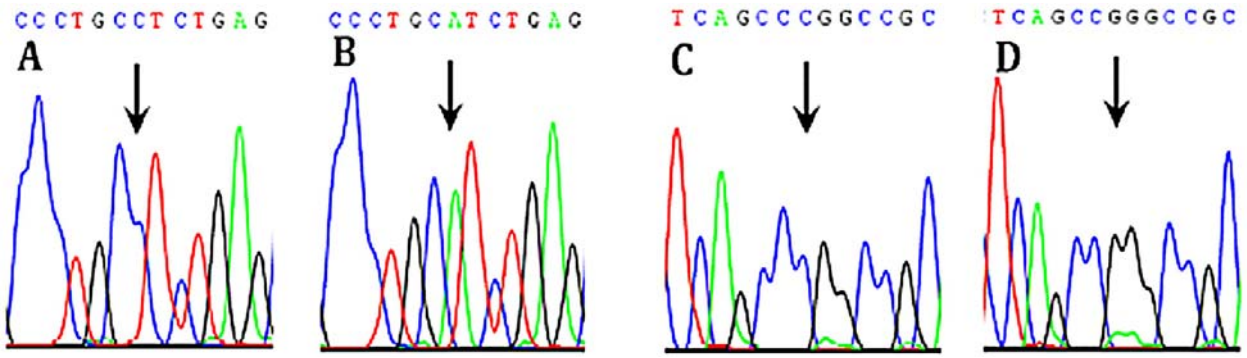

Figure 3. The sequences of MET promoter region in different clones of rhabdomyosarcoma cells. Nucleotide substitutions at sites -304C>A (A and B) and +206C>G (C and D). The arrows mark the sites of substitutions.

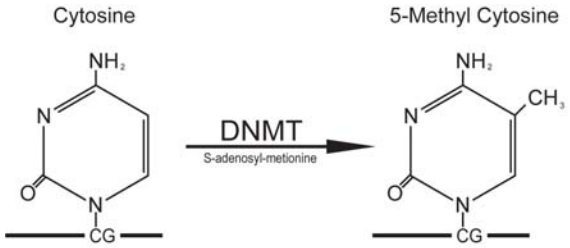

Figure 4. Schematic representation of the process of cytosine methylation.

further investigated to assess their relevance for the MET transcriptional regulation.

Although a growing number of reports delivers a vast amount of data, the results from disparate studies are often contradictory, ambivalent or requiring further confirmation. Since the observed phenotypes are usually connected with numerous disparate traits, it is also conceivable that only the cumulative analysis of many contributing low-penetrance loci might provide significant associations with malignancy. In the future the methods of statistical analysis may possibly require unification among different studies.

5. DNA METHYLATION

Another significant aspect of gene regulation is DNA methylation. Its epigenetic character allows to change gene expression, without changing its sequence. Changes in DNA methylation pattern may be stable heritable attributes, but can also undergo alterations during the cell cycle**.** Normally DNA methylation helps to maintain an order in genetic machinery and its malfunction is an important step in carcinogenesis.

5.1. Process of DNA methylation

DNA methylation relies on covalent modification of cytosine ring, by an addition of methyl (CH3) group at 5th position, which occurs in cytosines that precede guanines, forming dinucleotide called CpG. Cytosine methylation may also occur in other dinucleotides like CpT or CpA (40). The process is catalyzed by a family of proteins called DNA methyltransferases DNMTs (Figure 4).

There are three known mammalian DNMTs: DNMT1 - responsible for maintenance of established methylation pattern, DNMT3a and DNMT3b which are providing *de novo* methylation of DNA (41). All three enzymes are crucial for normal run of the cell life functions. Mutations causing lack of activity of any of those are usually lethal (42).

A mechanism of methylation-dependent transcription suppression is still under investigation. One of the theories (Figure 5 panel B) assumes that -CH_3 adduct becomes a steric obstacle for potential DNA binding proteins (43). Second (Figure 5 panel C) predicts that methylated DNA is specifically recognized by methylated CpGs binding protein complexes (MeCP2, MeCP1), which limit access of transcription factors to regulatory sequences.

5.2. CpG islands

Distribution of CpG dinucleotides in mammalian genome is not random. Some of these CpG sites (ca.7% of all) are grouped into short stretches of DNA called CpG islands, which are often placed near or in transcription initiation sites (41). Roughly 50-60% of all genes contain some CpG islands.

Unlike as heavy methylated CpGs found in non promoter regions, CpG islands remain usually unmethylated (44, 45), however various exceptions have

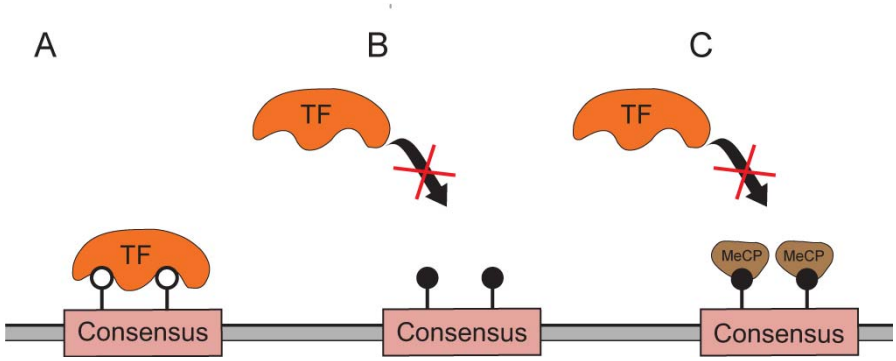

Figure 5. Methylation dependent transcription regulation. TF - transcription factor, MeCP1 and MeCP2 – protein complexes able to recognize methylated DNA. Filled dots – methylated CpGs, unfilled dots – unmethylated CpGs. A) TF bound to unmethylated consensus sequence. B) TF binding blocked by methylated cytosines. C) Methylated cytosines recognized by MeCP blocking acces for TF.

been reported (46). It is implied that CpG islands are initiation sites for both, replication and transcription of DNA (47).

5.3. Function of DNA methylation

There are various proposed functions of DNA methylation. Some already proved, some still theoretical. Methylation of CpGs in regulatory sequences like, promoters, enhancers or repressors, generally suppresses their function. Suppression of gene activity may be illustrated with example of methylation dependent parental gene imprinting phenomenon - an inheritance process independent of the classical Mendelian inheritance. Methylation dependent gene suppression is also important in maintenance of balanced expression of genes located on the X chromosome in female cells (48).

Methylation of non coding regions like a pericentromeric heterochromatin, helps to maintain proper structure and integrity of chromosome (49). Methylation has also been proposed as a defense system against mobile genetic elements like transposons (41, 43, 44).

5.4. Dynamics of methylation pattern and methylation polymorphism

Recent experiments revealed that methylation pattern can undergo cyclical and strand-specific modifications, during the cell cycle. For example, methylation status of the 5` proximal trefoil factor 1 (TFF1 also known as pS2) promoter site, in doxorubicin synchronized MCF-7 cells revealed cyclical changes, resulting in modified promoter activity (50). It is proven that DNA methyltranferases exhibit both methylating and demethylating activities during this process. Active demethylation was described as a reaction of deamination of 5m-CpG dinucleotides (50, 51).

5.5. Aberrant DNA methylation in cancer cells

It was assumed that epigenetic background of cancer development is connected with global DNA hypomethylation. This hypothesis linked loss of pericentromeric DNA methylation with chromosomes destabilization and retrotransposones reactivation (52).

Rapid development of methylation analysis techniques allowed more specific approach to DNA methylation matters. There are various reports of hypomethylation dependent protooncogenes activation like cMYC or H-RAS (53), or serin protease inhibitor (maspin) gene in colon cancer cells (54). Promoter demethylation was also reported in breast cancer tissues for N-acetyltransferase 1 gene, causing strong overexpression of this gene as compared with normal cells (55). Our research also reveals lowered methylation of MET promoter in rhabdomyosarcoma cell lines in comparison to leukocytes of healthy individuals (Figure 6).

Another type of methylation related cancerogenesis, is gene specific promoter hypermethylation. These affects promoters of tumor suppressing genes (TSG). These include genes connected with cell cycle regulation like p16, p15 or Rb, genes responsible for apoptosis – DAPK1 (calmoduline dependent enzyme with kinase activity) or DNA repair machinery BRCA1 (gene involved in DNA repair and transcription activation) (56, 57, 58).

Hypermethylation is also connected with various leukemias. It was reported that p15, or calcitonin gene were hypermethylated in 65% of myelodysplastic syndromes. Also calcitonin gene was found as hypermethylated in 95% of acute leukemias (59).

5.6. Methylation in diagnostic and therapeutic aspect

A rapid extension of knowledge about connection between methylation status and cancer development, is opening new possible ways of tumor detection or even treatment.

Some recent methods of methylation analysis, like bisulfite sequencing, or bisulfite specific PCR (BSP), allows us to detect changes in single CpG dinucleotide, what may be helpful in prognosis and subclasification of tumor cells.

Therapeutic aspect of DNA methylation is linked with fact that all epigenetic changes are reversible, and it

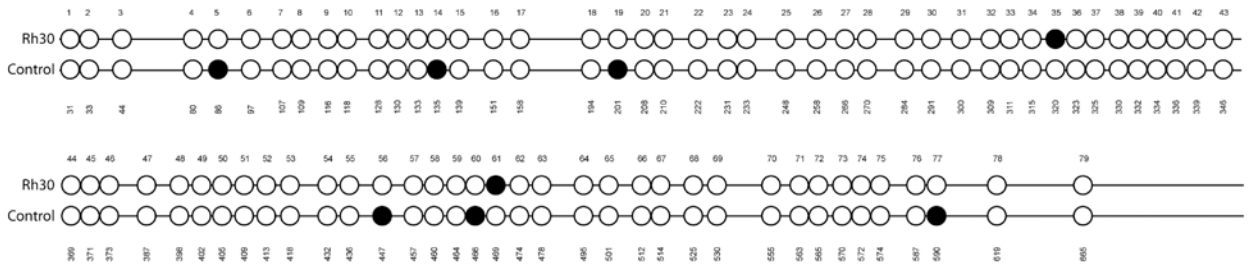

Figure 6. Schematic presentation of MET promoter methylation in rhabdomyosarcoma (RH30) sample. Filled dots – methylated CpGs, unfilled dots – unmethylated CpGs.

can be alterated with chemical substances. Drugs like: decitabine (5-aza-2`-deoxycytidine), 5-azacytydine or dihydro-5-azacytidine are effective methylation inhibitors, commonly in use to prevent silencing of tumor suppressors (60). On the other hand, it must be noticed that alteration of methylation may cause serious side effects, including tumor stimulation. It has been reported that pancreatic cancer cells treated with 5-aza-2-deoxycytidine showed substantial activity of metalloproteinase genes, which are typical for invasive phenotype (61).

Concluding, it seems that analysis of DNA methylation can be useful diagnostic factor, helping to diagnose early phase of tumor development. Pharmaceutical modulation of methylation status can improve anticancer therapy to make it more efficient. However, threat of potential side effects forces to continue research on methylation phenomenon.

6. TRANSCRIPTIONAL CONTROL

Transcription factors control the rate at which a given gene is transcribed. They can be classified according to their mechanistic properties (general/basal, upstream, inducible), function (constitutively active, conditionally active-developmental or signal dependent), and on the basis of their DNA binding domains (basic elements domains-basic-helix-loop-helix, zinc-coordinating DNA-binding domains, helix-turn-helix, beta-scaffold factors with minor groove contacts and other transcription factors) (62, 63, 64, 65). Transcription factors undoubtedly play the vital role in a wide variety of cellular processes and alterations in these factors can result in human disease. It is shown that disorders of hormone response and developmental defects originate from mutational inactivation of specific transcription factors. Finally, mutations in oncogenes which encode transcription factors can cause their overexpression and lead to cancer (65).

Each of these steps involves upregulation or downregulation of specific genes. Apart from high-penetrance genetic mutations, some much more subtle, low-penetrance changes are capable of influencing the disease progression and treatment. Virtually any change within the transcription regulatory elements can be of significance to the cellular homeostasis, since it may alter transcription factor binding patterns and cause differential gene expression.

6.1. Significance of transcription control in tumorigenesis and cancer progression

In the course of malignant progression cancer cells have to overcome certain 'safety' barriers guarding the organism homeostasis. Hanahan and Weinberg (2000) list six crucial alterations that a cell has to undergo in order to develop fully malignant phenotype. These traits include: self-sufficiency with respect to growth signals, unresponsiveness to factors blocking proliferation, inhibition of apoptosis, acquisition of limitless replicative potential, stimulation of angiogenesis and ability to form metastases by invasion of surrounding tissues (66).

A major goal in the field of cancer therapy is to define the mechanisms underlying transcriptional regulation. Some gene products are required for the survival of all cell types and their expression is often protected from environmental fluctuactions. Other genes are regulated by a consequence of changes in intracellular microenvironment (67). Cancer cells exhibit common functional characteristics. In the beginning of 20-th century Otto Warburg discovered that cancer cells have higher rates of glycolysis then normal cells. Yeung *et al.* (2008) (68) states that it is a result of mitochondrial changes, upregulation of rate-limiting enzymes in glycolysis, changes in intracellular pH regulation, loss of p53 function resulting in reprogramming from oxidative phosphorylation to glycolytic metabolism induced by hypoxia. The regulation of metabolism depends mainly on c-MYC, HIF-1 and p53 transcription factors. Process of oncogenic changes involve gene deletions, amplifications, mutations and many oncogenes and tumor suppressor genes cluster along the signaling pathways regulating c-MYC, HIF-1 and p53 (68). Poor outcome in invasive breast cancer correlates with strong HIF-2alpha expression and HIF-2alpha was found to be a strong independent prognostic marker in breast cancer (69). There are data indicating that constitutive expression of the c-MYC is present in the majority of human tumors (70). C-MYC directly regulates the expression of AP4, which is a direct transcriptional target of c-MYC. AP4 initially was shown to activate transcription, but more recent studies indicates that AP4 also repress viral and cellular genes. AP4 is specifically expressed in colon progenitor and colorectal carcinoma cells. C-MYC influences AP4 to maintain cells in proliferative, progenitor-like state (70).

Deregulation of another group of transcription factors is involved in melanoma development. Acquisition of the metastatic phenotype is caused by the loss of Activator Protein 2alpha (AP-2alpha) and upregulation in expression of cAMP-responsive element binding protein/Activating Transcription Factor-1 (CREB/ATF-1) family of transcription factors. Upregulation was also observed of ATF-2, SNAIL/SLUG, NF-kappaB, STAT3 and 5. NF-kappaB activation is also a selective advantage for colorectal tumor development (71). All these changes result in aberrant adhesive characteristics, matrix degrading enzymes and other changes in interaction of metastatic cells with the extracellular milieu. These effects can be greatly inhibited by altering the activity of mentioned above transcription factors (72).

It is also shown that the expression level of AP1, STAT3 and DBP (albumin D-box) transcription factors differ between androgen-dependent and androgen-independent prostate cancer patients (72) and AP1 is documented to be associated with progression and recurrence of prostate cancer (73).

The significance of transcription control in tumorigenesis and cancer progression is described below on the example of MET receptor, which is crucial in cancerogenesis and metastasis.

6.2. Role of human MET receptor in tumorigenesis

MET receptor and its ligand, hepatocyte growth factor (HGF) is an important player in mitogenesis, morphogenesis and acquisition of migratory phenotype (74). HGF/MET axis plays its role in neoplastic transformation through activation of key oncogenic pathways and influencing dissociation ability of cells leading to metastasis (75, 76). It plays also an important role in metastasis itself (39, 77). Overexpression of MET receptor has been observed in a variety of tumors including: papillary renal carcinoma, musculoskeletal tumor, adenocarcinoma (78, 79, 80, 81).

6.3. Regulation of MET expression

Human MET receptor promoter region spans approximately 800 bp of DNA. There are -297 bp fragment immediately upstream to the transcription initiation site followed by 5'untranslated region up to +358 bp. The fragment within 600 bp around transcription start site has more then 70% of G-C content. MET promoter sequence lacks TATA and CCAAT (similar to TATA-box base core promoter component) elements, which is a common feature in promoters of several tyrosine kinase receptors, such as insulin receptor, EGF, RET, UFO. The -297 bp fragment contains a number of potential regulatory elements, including AP1, AP2, NF-κB, IL-6RE (81, 82). There are multiple GC boxes in the promoter region of MET which are binding sites for Sp family of transcription factors (81, 82, 83). There are at least four members of Sp family (Sp1-Sp4) and Sp1, mentioned earlier, is the prototype of this family. It activates transcription for a large number of genes. Sp1 and Sp3 are often present in the same cell and have similar DNA binding specificity. It was shown that Sp1 and Sp3 proteins (Sp1-95 kDa and two different isoforms of Sp3-124 kDa or 84 kDa derived from distinct internal translation initiations) bind to the promoter region of the MET and functionally activate MET transcription in all types of renal cells. Conversely, deprivation of Sp proteins and blocade of Sp binding by chemical antagonist inhibits MET expression in renal epithelial cells (83). It was shown that Sp1 interacts also with other transcription factors. Sp family binding site is adjacent to AP1 site. They are separated by approximately 30 bp. There are evidences that Sp binding reduces AP1 binding to the MET promoter. This inhibition may be caused by steric interaction (83).

6.4. Regulation of MET expression in cancer

6.4.1. Upregulation

Formation of several human tumors and malignant progression are associated with changes in expression of HGF and its receptor MET. It was shown that Sp1 was markedly overexpressed in many fibrosarcoma cell lines in comparison to normal fibroblast cells. Deletion analysis of site-directed mutagenesis of the MET promoter indicated that SP1 sites are critical for transcription of MET and Sp1 can be used to control the level of MET expression (84).

Most tumors are able to develop their own blood vessels, however its vasculature is irregular and delivery of oxygen is inefficient. There are evidences that hypoxia induces the expression of MET protein in several types of tumors (cervical carcinoma, breast carcinoma, pancreatic cancer) and MET is especially upregulated in hypoxic regions of tumors (79, 85, 86). Hypoxic conditions activates MET promoter which contains several HIF-1 binding sites in sense and antisense orientation. Analysis of the promoter shown that hypoxia-responsive region has 264 bp. It contains AP1 site and two HIF-1 binding sites (HBS-4 and HBS-5). Mutagenesis of these sites caused reduction of transcriptional response to hypoxia or exogenous HIF-1. Similar effect was obtained for mutagenesis of AP1 site and it also impaired the basal activity of the promoter, which confirm very important role of AP1 in controlling MET transcription (79). Induction of MET expression under hypoxic conditions was determined also in glioma cells. Transfection of siRNA against HIF-1 alpha abrogated the induction of MET (86). Recently Kitajima *et al* (2008) showed that HIF-1 has another target gene – hepatocyte growth factor activator (HGFA). HGFA converts HGF into its active form. Experiments with HGFA siRNA under hypoxia shown HIF-1 alpha dependent induction of HGFA. HGF/HGFA/MET interactions might play a central role in the invasiveness of pancreatic cancer (85).

Interestingly, it was also documented that induction of MET expression by hypoxia may play an important role in physiology of early pregnancy. One of the important steps during early pregnancy is the invasion of trophoblast cells, which are exposed to low-oxygen conditions, into the deciduas of the uterus. Low oxygen tension stimulated the expression of MET mRNA and protein. Studies with chromatin immunoprecipitation assay revealed that this upregulation of MET was induced directly by HIF-1alpha (86, 87).

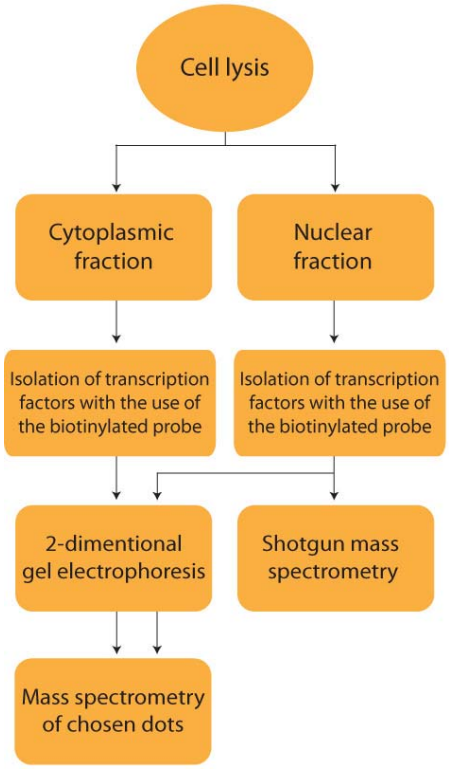

Figure 7. Schematic representation of pending and planned experiments in fishing and sequencing of transcription factors.

Experimental data suggest that MET expression in melanoma cells is regulated by microphthalmia-associated transcription factor (Mitf) which was known as the factor helping to control the development and function of pigment-producing cells (melanocytes). Disruption of Mitf blocked HGF-dependent increases in MET mRNA and protein. It was shown that MET is a direct transcriptional target gene for Mitf (88, 89). Other researchers found that autonomous activation of the melanocortin receptor-1 (MCR-1) is also responsible for MET upregulation in melanoma cells (90).

In addition to above mentioned factors upregulating MET receptor, the region between 300 and 840 bp upstream to the transcription start site contains four binding sites for members of Ets transcription factor family, known to involve in invasive growth. It suggests that Ets factors promote Met transcription contributing to the invasive phenotype (91).

6.4.2. Downregulation

There are several factors involved in downregulation of MET expression. One of them is interferon (IFN)-alpha. Analysis of primary human hepatocytes shown that IFN-alpha suppresses MET promoter through down-regulation of Sp1 binding. It results in decreased HGF-induced signals and lower cell proliferation (92).

Another factor engaged in MET downregulation is phosphatase and tensin homolog (PTEN). PTEN is a tumor suppressor and together with MET co–regulate many genes involved in cell growth regulation, such as transcription and growth factors, ubiquitin and oncogenic cell signaling pathways. Their interactions are not well understood yet, however experiments with expression microarrays demonstrated that PTEN downregulated MET and prevent uncontrolled cell growth that can lead to the formation of tumors (93).

Recently, Daxx was identified as a transcription repressor of MET. Daxx binds to the MET promoter and repress transcription in mouse cells. There was also suggested repressive potential of Daxx in cancer progression in cancer cell lines and metastatic breast cancer specimens (94).

Experiments revealed that the MET promoter contains one putative binding site for HES proteins, which are helix-loop-helix type of transcriptional repressor known to be a downstream target of Notch. It was demonstrated that HES-1 binds to MET promoter. Moreover, Hes-1 is sufficient to induce MET downregulation. On the other hand, MET activation induces Notch signaling, which through Hes-1 represses MET transcription. There is a negative feedback regulation where MET activation causes induction of Noch function, which in turn downregulates HGF activity by repression of MET (95).

6.5. Preliminary data in isolation of MET promoter transcription factors

A biotinylated probe used in the experiments was a fragment of MET promoter DNA labeled with biotin. The probe is expected to bind the transcription factors present in cell lysates. Streptavidin beads bound to magnets and added to the mixture allowed to separate on magnetic columns the proteins coupled to biotin labeled probe. Figure 7. presents the outline of isolation and sequencing of transcription factors.

Results of isolation procedure are presented on polyacrylamide gel separation and 2-dimensional gel electrophoresis (2-DE) - Figure 8 A and B. Protein fishing analysis resulted in differences in bands profile between cytoplasmic and nuclear samples of rhabdomyosarcoma cell line. The dots visualised in 2-DE electrophoresis are possible transcription factors being under investigation. They are located on the gel in the area of neutral pH (pH 6-8) and a size between 40 and 120 kDa.

7. CONCLUSIONS

In this article we have tried to provide an overview of the regulation of transcription in cancer. We have attempted to show that genetic variations of the promoter region of the particular gene, even commonly present in the population, may lead to disturbances in mismatch repair system, proliferation controling and apoptosis, modified cell adhesion or matrix degradation. They contribute to higher risk of developing certain

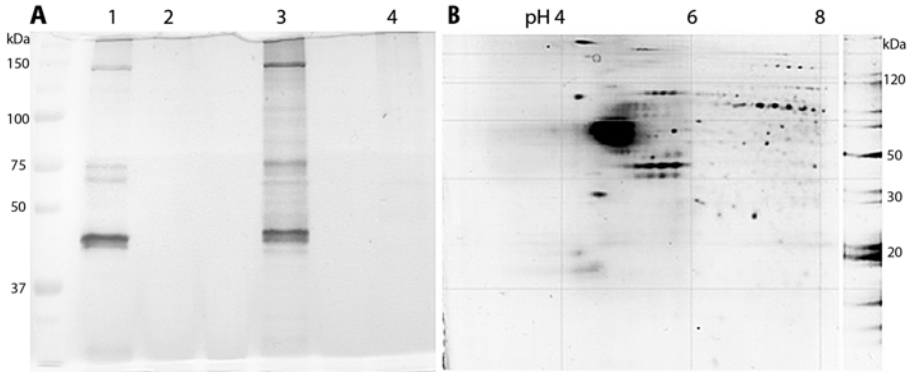

Figure 8. Results of isolation of transcription factors from cytoplasmic and nuclear samples of rhabdomyosarcoma cell line presented on polyacrylamide gel separation -(A) and 2-dimensional gel electrophoresis (2-DE) -(B). (A) 1: cytoplasmic fraction with probe, 2: cytoplasmic fraction without probe, 3: nuclear fraction with probe, 4: nuclear fraction without probe. This figure represents own unpublished data.

cancers, sufferring from faster progression to malignant stage and differentially responding to the applied therapy.

Epigenetic regulation of transcription by aberrant DNA methylation is another factor contributing to cancer development. Not only global DNA hypomethylation, which was first assumed to be epigenetic background of cancer development, but also gene specific promoter hypermethylation is related to cancerogenesis.

Finally, any change within the transcription regulatory elements can be of significance to the cellular homeostasis. Deregulation of transcription factors is involved in many types of cancers. Their upregulation and activation or constitutive expression are involved in pro-oncogenic mechanisms in the majority of human tumors.

Only the cumulative effect of promoter polymorphisms, abberant DNA methylation and changes in transcription factors expression, rather than the single effect, should be related to pathogenic states or cancerogenesis.

8. REFERENCES

1. EM Blackwood, JT Kadonaga: Going the Distance: A Current View of Enhancer Action. *Science* 281, 60-63 (1998)

2. JEF Butler, JT Kadonaga: The RNA polymerase II core promoter: a key component in the regulation of gene expression. *Genes Dev* 16, 2583–2592 (2002)

3. T Juven-Gershon, JY Hsu, JT Kadonaga: Perspectives on the RNA polymerase II core promoter. *Biochem SocTran* 34 (6), 1047-1049 (2006)

4. T Juven-Gershon, JY Hsu, JWM Theisen, JT Kadonaga: The RNA polymerase II core promoter — the gateway to transcription. *Curr Opin Cell Biol* 20, 253–259 (2008)

5. C Yang, E Bolotin, T Jiang, FM Sladek, E Martinez: Prevalence of the Initiator over the TATA box in human and yeast genes and identification of DNA motifs enriched in human TATA-less core promoters. *Gene* 389 (1), 52–65 (2007)

6. J Schug, WP Schuller, C Kappen, JM Salbaum, M Bucan, CJJ Stoeckert: Promoter features related to tissue specificity as measured by Shannon entropy. *Genome Biol* 6(4):R33 (2005)

7. P Carninci, A Sandelin, B Lenhard, S Katayama, K Shimokawa, J Ponjavic, CA Semple, MS Taylor, PG Engström, MC Frith, AR Forrest: Genome-wide analysis of mammalian promoter architecture and evolution. *Nat Genet* 38 (6), 626-635 (2006)

8. ND Trinklein, SF Aldred, SJ Hartman, DI Schroeder, RP Otillar, RM Myers: An abundance of bidirectional promoters in the human genome. *Genome Res* 14 (1), 62-66 (2004)

9. SJ Cooper, ND Trinklein, ED Anton, L Nguyen, RM Myers: Comprehensive analysis of transcriptional promoter structure and function in 1% of the human genome. *Genome Res* 16 (1), 1-10 (2006)

10. MC Thomas, CM Chiang: The General Transcription Machinery and General Cofactors. *Crit Rev Biochem Mol Biol* 41, 105-178 (2006)

11. S Hahn: Structure and mechanism of the RNA polymerase II transcription machinery. *Nat Struct Mol Biol* 11(5), 394-403 (2004)

12. RD Kornberg: The molecular basis of eukaryotic transcription. *Proc Natl Acad Sci USA* 104 (32), 12955–12961 (2007)

13. A Wärnmark, E Treuter, APH Wright, JÅ Gustafsson: Activation Functions 1 and 2 of Nuclear Receptors: Molecular Strategies for Transcriptional Activation. *Mol Endocrinol* 17 (10), 1901-1909 (2003)

14. WS Samowitz, K Curtin, RK Wolff, H Albertsen, C Sweeney, BJ Caan, CM Ulrich, JD Potter, ML Slattery:

The MLH1 -93 G>A promoter polymorphism and genetic and epigenetic alterations in colon cancer. *Genes Chromosomes Cancer* 47 (10), 835-44. (2008)

15. I Harley, B Rosen, HA Risch, K Siminovitch, ME Beiner, J McLaughlin, P Sun, SA Narod: Ovarian cancer risk is associated with a common variant in the promoter sequence of the mismatch repair gene MLH1. *Gynecol Oncol* 109 (3), 384-387 (2008)

16. ME Beiner, BRosen, A Fyles, I Harley, T Pal, K Siminovitch, S Zhang, P Sun, SA Narod: Endometrial cancer risk is associated with variants of the mismatch repair genes MLH1 and MSH2. *Cancer Epidemiol Biomarkers Prev* 15 (9), 1636-1640 (2006)

17. E Thompson, RL Dragovic, SA Stephenson, DM Eccles, IG Campbell, A Dobrovic: A novel duplication polymorphism in the FANCA promoter and its association with breast and ovarian cancer. *BMC Cancer* 5 (1), 43 (2005)

18. GL Bond, W Hu, EE Bond, H Robins, SG Lutzker, NC Arva, J Bargonetti, F Bartel, H Taubert, P Wuerl, K Onel, L Yip, SJ Hwang, LC Strong, G Lozano, AJ Levine: A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumot supressor pathway and accelerates tumor formation in humans. *Cell* 119, 591-602 (2004)

19. S Cattelani, R Defferrari, S Marsilio, R Bussolari, O Candini, F Corradini, G Ferrari-Amorotti, C Guerzoni, L Pecorari, C Menin, R Bertorelle, P Altavista, HP McDowell, R Boldrini, C Dominici, GP Tonini, G Raschellà, B Calabretta: Impact of a single nucleotide polymorphism in the MDM2 gene on neuroblastoma development and aggressiveness: results of a pilot study on 239 patients. *Clin Cancer Res* 14 (11), 3248-3253 (2008)

20. K Asomaning, AE Reid, W Zhou, RS Heist, R Zhai, L Su, EL Kwak, L Blaszkowsky, AX Zhu, DP Ryan, DC Christiani, G Liu: MDM2 Promoter Polymorphism and Pancreatic Cancer Risk and Prognosis. *Clin Cancer Res* 14 (12), 4010-4015 (2008)

21. H Hirata, Y Hinoda, N Kikuno, K Kawamoto, Y Suehiro, Y Tanaka, R Dahiya: MDM2 SNP309 polymorphism as risk factor for susceptibility and poor prognosis in renal cell carcinoma. *Clin Cancer Res* 13 (14), 4123-4129 (2007)

22. S Wilkening, JL Bermejo, K Hemminki: MDM2 SNP309 and cancer risk: a combined analysis. *Carcinogenesis* 28 (11), 2262-2267 (2007)

23. HS Bachmann, F Otterbach, R Callies, H Nückel, M Bau, KW Schmid, W Siffert, R Kimmig: The AA genotype of the regulatory BCL2 promoter polymorphism (-938C>A) is associated with a favorable outcome in lymph node negative invasive breast cancer patients. *Clin Cancer Res* 13 (19), 5790-5797 (2007)

24. H Nückel, UH Frey, M Bau, L Sellmann, J Stanelle, J Dürig, KH Jöckel, U Dührsen, W Siffert: Association of a novel regulatory polymorphism (-938C>A) in the BCL2 gene promoter with disease progression and survival in chronic lymphocytic leukemia. *Blood* 109 (1), 290-297 (2007)

25. BPL Wijnhoven, M Pignatelli: E-cadherin-catenin: more than a "sticky ". *Lancet* 354, 356-357 (1999)

26. LC Li, RM Chui, M Sasaki, K Nakajima, G Perinchery, HC Au, D Nojima, P Carroll, R Dahiya: A single nucleotide polymorphism in the E-cadherin gene promoter alters transcriptional activities. *Cancer Res* 60 (4), 873-876 (2000)

27. G Wang, X Hu, C Lu, C Su, S Luo, ZW Luo: Promoter-hypermethylation associated defective expression of E-cadherin in primary non-small cell lung cancer. *Lung Cancer* 62 (2), 162-72 (2008)

28. GY Wang, CQ Lu, RM Zhang, XH Hu, ZW Luo: The E-cadherin gene polymorphism 160C->A and cancer risk: A HuGE review and meta-analysis of 26 case-control studies. *Am J Epidemiol* 167 (1), 7-14 (2008)

29. Y Shin, IJ Kim, HC Kang, JH Park, HR Park, HW Park, MA Park, JS Lee, KA Yoon, JL Ku, JG Park: The E-cadherin -347G->GA promoter polymorphism and its effect on transcriptional regulation. *Carcinogenesis* 25 (6), 895-899 (2004)

30. JL Rutter, TI Mitchell, G Butticè, J Meyers, JF Gusella, LJ Ozelius, CE Brinckerhoff: A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. *Cancer Res* 58 (23), 5321-5325 (1998)

31. G Ghilardi, ML Biondi, J Mangoni, S Leviti, M DeMonti, E Guagnellini, R Scorza: Matrix metalloproteinase-1 promoter polymorphism 1G/2G is correlated with colorectal cancer invasiveness. *Clin Cancer Res* 7 (8), 2344-2346 (2001)

32. A Hettiaratchi, NJ Hawkins, G McKenzie, RL Ward, JE Hunt, D Wakefield, N Di Girolamo: The collagenase-1 (MMP-1) gene promoter polymorphism - 1607/2G is associated with favourable prognosis in patients with colorectal cancer. *Br J Cancer* 96 (5), 783-792 (2007)

33. L Su, W Zhou, S Park, JC Wain, TJ Lynch, G Liu, DC Christiani: Matrix metalloproteinase-1 promoter polymorphism and lung cancer risk. *Cancer Epidemiol Biomarkers Prev* 14 (3), 567-570 (2005)

34. W Ju, S Kang, JW Kim, NH Park, YS Song, SB Kang, HP Lee: Promoter polymorphism in the matrix metalloproteinase-1 and risk of cervical cancer in Korean women. *Cancer Lett* 217 (2), 191-196 (2005)

35. EG Pearce, RC Laxton, AC Pereira, S Ye: Haplotype effects on matrix metalloproteinase-1 gene promoter activity in cancer cells. *Mol Cancer Res* 5 (3), 221-227 (2007)

36. A Loktionov: Common gene polymorphisms, cancer progression and prognosis. *Cancer Lett*, 208, 1-33 (2004)

37. Consortium ENCODE Project: Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. *Nature* 447 (7146), 799-816 (2007)

38. Consortium, The International HapMap: A second generation human haplotype map of over 3.1 million SNPs. *Nature* 449 (7164), 851-861 (2007)

39. K Jankowski, M Kucia, M Wysoczynski, R Reca, D Zhao, E Trzyna, J Trent, S Peiper, M Zembala, J Ratajczak, P Houghton, A Janowska-Wieczorek, MZ Ratajczak: Both hepatocyte growth factor (HGF) and stromal-derived factor-1 regulate the metastatic behavior of human rhabdomyosarcoma cells, but only HGF enhances their resistance to radiochemotherapy. *Cancer Res* 15, 63(22), 7926-35 (2003)

40. BH Ramsahoye, D Biniszkiewicz, F Lyko, V Clark, AP Bird, R Jaenisch: Non CpG methylation is prevalent in embryonic stem cells and may be mediated by DNA methyltransferase 3a. *Proc Natl Acad Sci* USA 97,5237-5242 (2000)

41. TH Bestor: The DNA methyltransferases of mammals. *Hum Mol Genet* 9, 2395–2402 (2000)

42. M Monk, M Boubelik, S Lehnert: Temporal and regional changes in DNA methylation in the embryonic, extraembryonic and germ cell lineages during mouse embryo development. *Development* 99, 371-82 (1987)

43. RJW O'Neill, MJ Oneill, JAM Graves: Undermethylation associated with retroelement activation and chromosome remodelling in an interspecific mammalian hybrid. *Nature* 393,68-72 (1998)

44. TH Bestor: The host defence function of genomic methylation patterns. *Novartis Found Symp* 214:187-95, discussion 195-9, 228-32 (1998)

45. A Bird: DNA methylation patterns and epigenetic memory. Genes Dev 16, 6–21(2002)

46. F Song, JF Smith, MT Kimura, AD Morrow, T Matsuyama, H Nagase, WA Held: Association of tissue-specific differentially methylated regions (TDMs) with differential gene expression. *Proc Natl Acad Sci USA* 102, 3336–3341 (2005)

47. S Delgado, M Go´mez, A Bird, F Antequera: Initiation of DNA replication at CpG islands In mammalian chromosomem. *EMBO J*.17 (8),2426–2435 (1998)

48. T Goto, M Monk: Regulation of X-chromosome inactivation in development in mice and humans. *Microbiol Mol Biol Rev* 62, 362-78 (1998)

49. M Ehrlich: DNA methylation: normal development, inherited diseases, and cancer. *J Clin Ligand Assay* 23,144-6 (2000)

50. S Kangaspeska, B Stride, R Métivier, M Polycarpou-Schwarz, D Ibberson, RP Carmouche, V Benes, F Gannon, G Reid: Transient cyclical methylation of promoter DNA**.** *Nature* 6, 452 (7183):112-5 (2008)

51. R Métivier, R Gallais, C Tiffoche, C Le Péron, RZ Jurkowska, RP Carmouche, D Ibberson, P Barath, F Demay, G Reid, V Benes, A Jeltsch, F Gannon, G Salbert: Cyclical DNA methylation of a transcriptionally active promoter. *Nature* 6, 452 (7183):45-50 (2008)

52. A.P Feinberg, B Tycko: The history of cancer epigenetics. *Nature Rev Cancer* 4, 1-11 (2004)

53. AP Feinberg, B Vogelstein: Hypomethylation of ras oncogenes in primary human cancers. *Biochem Biophys Res Commun* 111, 47-54 (1983)

54. N Nakamura, K Takenaga: Hypomethylation of the metastasis-associated S100A4 gene correlates with gene activation in human colon adenocarcinoma cell lines. *Clin Exp Metastasis* 16, 471–479 (1998)

55. SJ Kim, HS Kang, HL Chang, YC Jung, HB Sim, KS Lee, J Ro, ES Lee: Promoter hypomethylation of the N-acetyltransferase 1 gene in breast cancer. *Oncol Rep* 19 (3),663-8 (2008)

56. SV Harden, Y Tokumaru, WH Westra, S Goodman, SA Ahrendt, SC Yang, D Sidransky: Gene promoter hypermethylation in tumors and lymph nodes of stage I lung cancer patients. *Clin Cancer Re*s 9, 1370-1375 (2003)

57. C Stirzaker, DS Millar, CL Paul, PM Warnecke, J Harrison, PC Vincent, M Frommer, SJ Clark: Extensive DNA methylation spanning the Rb promoter in retinoblastoma tumors. *Cancer Res* 57, 2229-2237 (1997)

58. A Dobrovic, D Simpfendorfer: Methylation of the BRCA1 gene in sporadic breast cancer. *Cancer Res* 57, 3347-3350 (1997)

59. G Leone, L Teofili, MT Voso, M Lübbert: DNA methylation and demethylating drugs in myelodysplastic syndromes and secondary leukemias. *Haematologica* 87, 1324-1341 (2002)

60. J Goffin, E Eisenhauer: DNA methyltransferase inhibitors-state of the art. *Ann Oncol* 13, 1699-1716 (2002)

61. N Sato, N Maehara, GH Su, M Goggins. Effects of 5-aza-2´'-deoxycytidine on matrix metalloproteinase expression and pancreatic cancer cell invasiveness. *J Natl Cancer Inst* 9, 5327-330 (2003)

62. AH Brivanlou, JE Darnell: Signal transduction and the control of gene expression. *Science* 295 (5556), 813-8 (2002)

63. G Orphanides, T Lagrange, D Reinberg: The general transcription factors of RNA polymerase II. *Genes Dev* 10 (21), 2657-83 (1996)

64. P Stegmaier, AE Kel, E Wingender: Systematic DNA-binding domain classification of transcription factors. *Genome informatics International Conference on Genome Informatics* 15 (2), 276-86 (2004)

65. DS Lachman: Transcription Factors: An overview. *Int J Biochem Cell Biol* 29 (12), 1305-1312 (1997)

66. D Hanahan, RA Weinberg: The Hallmarks of Cancer. *Cell* 100, 57-70 (2000)

67. KW Scotto, DA Egan: Transcriptional regulation of MDR genes. *Cytotechnology* 27, 257–269 (1998)

68. SJ Yeung, J Pand, MH Leec: Roles of p53, Myc and HIF-1 in Regulating Glycolysis – the Seventh Hallmark of Cancer. *Cell Mol Life Sci* 65(24),3981-99 (2008)

69. K Helczynska, AM Larsson, L Holmquist Mengelbier, E Bridges, E Fredlund, S Borgquist, G Landberg, S Påhlman, K Jirström: Hypoxia-inducible factor-2alpha correlates to distant recurrence and poor outcome in invasive breast cancer. *Cancer Res* 68(22), 9212-20 (2008)

70. P Jung, A Menssen, D Mayr, H Hermeking: AP4 encodes a c-MYC-inducible repressor of p21. *Proc Natl Acad Sci USA* 105(39), 15046-51 (2008)

71. A Paradisi, C Maisse, A Bernet, MM Coissieux, M Maccarrone, JY Scoazec, P Mehlen: NF-kappaB regulates netrin-1 expression and affects the conditional tumor suppressive activity of the netrin-1 receptors. *Gastroenterology* 135(4), 1248-57 (2008)

72. VO Melnikova, M Bar-Eli: Transcriptional control of the melanoma malignant phenotype. *Cancer Biol Ther* 7(7), 997-1003 (2008)

73. X Ouyang, WJ Jessen, H Al-Ahmadie, AM Serio, Y Lin, WJ Shih, VE Reuter, PT Scardino, MM Shen, BJ Aronow, AJ Vickers, WL Gerald, C Abate-Shen: Activator protein-1 transcription factors are associated with progression and recurrence of prostate cancer. *Cancer Res* 1, 68(7), 2132-44 (2008)

74. OS Akbari, E Bae, H Johnsen, A Villaluz, D Wong, RA Drewell: A novel promoter-tethering element regulates enhancer-driven gene expression at the bithorax complex in the *Drosophila* embryo. *Development* 135, 123-131 (2008)

75. L Trusolino, PM Comoglio: Scatter-factor and semaphorin receptors: cell signalling for invasive growth. *Nat Rev Cancer* 2(4), 289-300 (2002)

76. E Lesko, M Majka: The biological role of HGF-MET axis in tumor growth and development of metastasis. *Front Biosci* 1, 13, 1271-80 (2008)

77. R Dagher, L Helman: Rhabdomyosarcoma: an overview. *Oncologist* 4 (1), 34-44 (1999)

78. M Jeffers, L Schmidt, N Nakaigawa, C Webb, G Weirich, T Kishida, B Zbar, G Vande Woude: Activating mutations for the Met tyrosine kinase receptor in human cancer. *Proc Natl Acad Sci USA* 94, 11445–11450 (1997)

79. V Wallenius, M Hisaoka, K Helou, G Levan, N Mandahl, JM Meis-Kindblom, LG Kindblom, JO Jansson: Overexpression of the Hepatocyte Growth Factor (HGF) Receptor (Met) and Presence of a Truncated and Activated Intracellular HGF Receptor Fragment in Locally Aggressive/Malignant Human Musculoskeletal Tumor. *Am Jf Pathol* 156, 821-829 (2000)

80. T Furukawa, WP Duguid, M Kobari, S Matsuno, MS Tsao: Hepatocyte growth factor and Met receptor expression in human pancreatic carcinogenesis. *Am J Pathol* 147(4), 889–895 (1995)

81. S Pennacchietti, P Michieli, M Galluzzo, M Mazzone, S Giordano, PM Comoglio: Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. *Cancer Cell* 3(4), 347-61 (2003)

82. G Gambarotta, S Pistoi, S Giordano, PM Comoglio, C Santoro: Structure and inducible regulation of the human MET promoter. *J Biol Chem* 29, 269(17), 12852-7 (1994)

83. X Zhang, Y Li, C Dai, J Yang, P Mundel, Y Liu: Sp1 and Sp3 transcription factors synergistically regulate HGF receptor gene expression in kidney. *Am J Physiol Renal Physiol* 284(1), F82-94 (2003)

84. H Liang, S O'Reilly, Y Liu, R Abounader, J Laterra, VM Maher, JJ McCormick: Sp1 regulates expression of MET, and ribozyme-induced down-regulation of MET in fibrosarcoma-derived human cells reduces or eliminates their tumorigenicity. *Int J Oncol* 24(5), 1057-67 (2004)

85. Y Kitajima, T Ide, T Ohtsuka, K Miyazaki: Induction of hepatocyte growth factor activator gene expression under hypoxia activates the hepatocyte growth factor/c-Met system via hypoxia inducible factor-1 in pancreatic cancer. *Cancer Sci* 99(7), 1341-7 (2008)

86. C Eckerich, S Zapf, R Fillbrandt, S Loges, M Westphal, K Lamszus: Hypoxia can induce c-Met expression in glioma cells and enhance SF/HGF-induced cell migration. *Int J Cancer* 15, 121(2),276-83 (2007)

87. M Hayashi, M Sakata, T Takeda, M Tahara, T Yamamoto, Y Okamoto, R Minekawa, A Isobe, M Ohmichi, K Tasaka, Y Murata: Up-regulation of c-met protooncogene product expression through hypoxia-inducible factor-1alpha is involved in trophoblast invasion under low-oxygen tension. *Endocrinology* 146(11), 4682-9 (2005)

88. GG McGill, R Haq, EK Nishimura, DE Fisher: c-Met expression is regulated by Mitf in the melanocyte lineage. *J Biol Chem*, 14, 281(15), 10365-73 (2006)

89. L Beuret, E Flori, C Denoyelle, K Bille, R Busca, M Picardo, C Bertolotto, R Ballotti: Up-regulation of MET expression by alpha-melanocyte-stimulating hormone and MITF allows hepatocyte growth factor to protect melanocytes and melanoma cells from apoptosis. *J Biol Chem* 11, 282(19), 14140-7 (2007)

90. G Elia, Y Ren, P Lorenzoni, R Zarnegar, MM Burger, D Rusciano: Mechanisms regulating c-met overexpression in liver -metastatic B16-LS9 melanoma cells. *J Cell Biochem* 81(3), 477-87 (2001)

91. G Gambarotta, C Boccaccio, S Giordano, M Ando, MC Stella, PM Comoglio: Ets up-regulates Met transcription. *Oncogene* 13, 1911-1917 (1996)

92. S Radaeva, B Jaruga, F Hong, WH Kim, S Fan, H Cai, S Strom, Y Liu, O El-Assal, B Gao: Interferon-alpha activates multiple STAT signals and down-regulates c-Met in primary human hepatocytes. *Gastroenterology* 122(4), 1020-34 (2002)

93. R Abounader, T Reznik, C Colantuoni, F Martinez-Murillo, EM Rosen, J Laterra: Regulation of c-Met-dependent gene expression by PTEN. *Oncogene* 9, 23(57), 9173-82 (2004)

94. VM Morozov, NA Massoll, OV Vladimirova, GG Maul, AM Ishov: Regulation of c-met expression by transcription repressor Daxx. *Oncogene* 3, 27(15), 2177-86 (2008)

95. MC Stella, L Trusolino, S Pennacchietti, PM Comoglio: Negative feedback regulation of Met-dependent invasive growth by Notch. *Mol Cell Biol* 25(10), 3982-96 (2005)

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