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Review

The significance of DNA methylation profile in metastasis-related genes for the progression of colorectal cancer

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Abstract: DNA methylation, an epigenetic modification plays a role in the pathogenesis of colorectal cancer (CRC). CRC cases, both sporadic and familial, are often characterized by abnormal pattern of the cytosine methylation in CpG dinucleotides in regulatory regions of genes important for cancer transformation. Also genes mutated in CRC can have their epigenetic pattern altered and we suggest that changes in DNA methylation array can be important for CRC metastatic potential – the main reason of CRC-associated mortality. These genes are: *KRAS,* genes of the Rho family of GTPases, *MACC1, Met, MTA1* and *RASSF1A.* In addition, genes encoding miRNA important for epithelial mesenchymal transition and other metastasis-related effects, such as mir-9, miR-34 and miR-210 can be good candidates for associating their DNA methylation profiles with CRC metastasis. Analysis of DNA methylation profile in various stages of CRC along with other genetic/epigenetic changes specific for all main stages of CRC transformation could help in anti-metastatic therapy immediately after CRC diagnosis. However, targeting DNA methylation pattern in CRC therapy is a conception, which requires further work to precisely change DNA methylation array, without affecting genes, whose expression should not be changed.

Key words: Colorectal cancer; CpG dinucleotide; DNA methylation; Epigenetic modification; Invasion; Metastasis.

Introduction

Colorectal cancer (CRC) has on average one of the best if not the best treatment outcome among all cancers if it is early detected and resected. However, even early detection and radical surgery can sometimes result in a delayed disease recurrence associated with metastasis. Therefore, it is important to identify a subset of early-detected and low-staging CRC cases, which might have a high metastatic potential. Because genetic aspects of CRC pathogenesis, both sporadic and familial, are relatively well known, they should be further explored to establish genetic markers and therapeutic strategies for CRC cases with a high metastatic potential. Also epigenetic modifications are considered to play an important role in CRC induction and development. Among them, DNA methylation seems to be of a special diagnostic and therapeutic significance as this kind of epigenetic modifications can be relatively easily detected and targeted. Therefore, determining the DNA methylation profile in genes which can be involved in CRC progression represents a pathway to establish early markers of CRC cases with a high metastatic potential.

Role of DNA methylation in gene expression and cancer transformation

DNA methylation, the addition of methyl groups to DNA residues, can play a role in normal cellular signaling or contribute to mutagenesis. Chemically, DNA methylation can occur at DNA bases or phosphotriesters, but the latter play a minor role in DNA mutagenesis and regulation of gene expression. Therefore, the term "DNA methylation" will refer to methylation of the DNA bases, which can be methylated enzymatically or by the action of methylating agents. Enzymes involved in DNA methylation are DNA methylatransferases and can be divided into two groups: enzymes methylating *de novo* DNA and enzymes responsible for the maintenance of the methylation pattern, which is primarily established in early embryogenesis by Dnmt3a and Dnmt3b DNA methyltransferases (1, 2). It is not clear, whether all enzymes involved in DNA methylation are known. Dnmt2 has been identified recently, but it is probably not involved in cytosine methylation essential for gene expression regulation. Moreover, proteins, with no enzymatic properties can be involved and the exact mechanism of action of Dnmt1 and Dnmt3a/3b is not fully known. In particular, it is not known whether methylation *de novo* affects either both strands simultaneously or it is limited to one strand. Therefore, the state of DNA methylation presented in figures further is only illustrative and may not reflect actual situation. More than 90% of methylated DNA bases in humans are cytosines, methylated within the CpG dinucleotide (3-5). Such dinucleotides occur with a much higher frequency in potentially transcriptionally active regions of the human genome and influence the expression of genes located in these regions. When the CpG sequence in a double-stranded DNA is methylated in one strand only

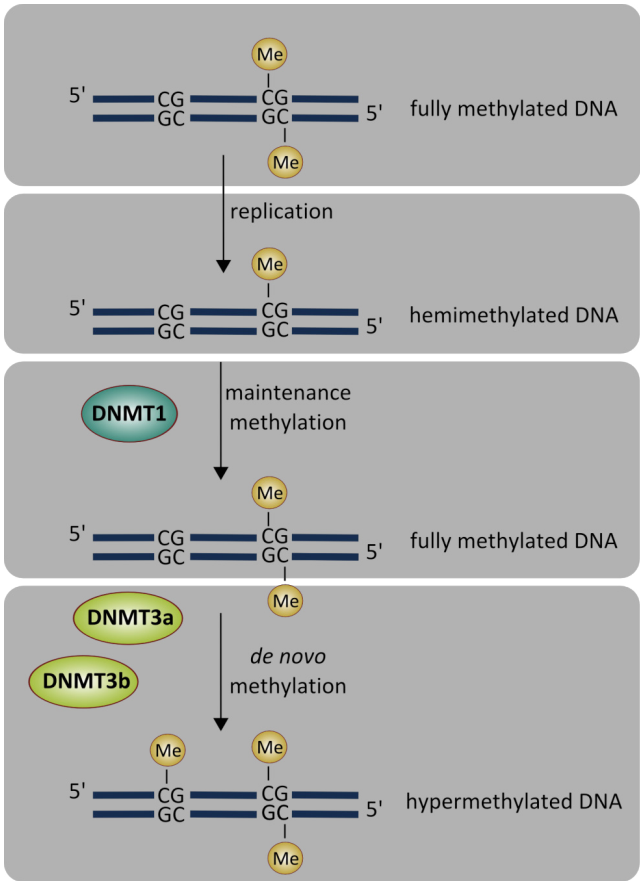

Figure 1. DNA methylation and demethylation.

(hemimethylated DNA), it is targeted by the Dnmt1 DNA methyltransferase, which methylate C in the complimentary CpG dinucleotide (Fig. 1).

DNA methylation occurs by the addition of a methyl group by *de novo* (DNMT3a/b) or by maintenance (DNMT1) DNA methyltransferases. Enzymatic DNA demethylation is associated with the ten-eleven translocation (TET) protein family, activation-induced cytidine deaminase (AID) and the action of base excision repair machinery, including DNA uracil glycosylases: TDG and SMUG. Exact mechanism of both *de novo* and maintenance methylation is not completely known, especially when it occurs in cancer. This process can be carried out but yet undiscovered proteins and result in asymmetric methylation of DNA strands, so the figure presents only one possible situation.

DNA methylation profile can be also regulated by active demethylation, which can result from the action of TET enzymes and DNA repair processes (6, 7).

DNA methylation plays an important role in the regulation of gene expression. This role is implemented by changes in chromatin structure, resulting in altered accessibility of transcription factors to genes, which are to be expressed. A methylated CpG island can be recognized by specific proteins, which recruit chromatin remodeling proteins (Fig. 2).

De novo DNA methylation conducted by DNA methyltransferases (DNMT3a/b) recruits the methyl-binding domain (MBD) proteins which prevent association of transcription factors to DNA thus blocking transcription and promote the recruitment of other proteins to convey gene expression silencing. "Ac" symbolizes acetylation of *N*-terminal tails of histones. *De novo* methylation is arbitrary presented in Fig. 2 to result in one strand methylation, which is probably only one of possibilities.

Since cancer transformation is associated with an abnormal gene expression pattern, changes in chromatin structure are required for this process. Although these changes can result from various mechanisms, altered DNA methylation pattern was reported to play a role in many cancers, including colorectal cancer (CRC) (8-12). This effect was observed in all stages of CRC, including first, clinically detectable, pre-cancerous lesions (13). Aberrant DNA methylation pattern in CRC is expressed mainly by hypermethylation of the CpG islands in the promoters of genes in the signaling pathways important for CRC development. Spectra of genes mutated and hypermethylated in CRC significantly overlap and hypermethylation in the promoter can occur more frequently than mutations in that region (14). However, DNA methylation should be considered in the context of other epigenetic changes in CRC, especially covalent histone modifications (15).

Early diagnosis in CRC, as in other cancers, is a *sine qua non* condition for a successful therapy. However, despite a recent progress, 1 out of 4 patients still presents with its metastatic form (16). The majority (80%) of CRCs are diagnosed as stages I–III, in which surgical resection can be attempted with good results. At stages I and II the 5-year average relative survival after surgery alone is 90%, but in the cases of spreading to the regional lymph nodes (stage III), it decreases to 70%. In the fraction of patients who are diagnosed once their

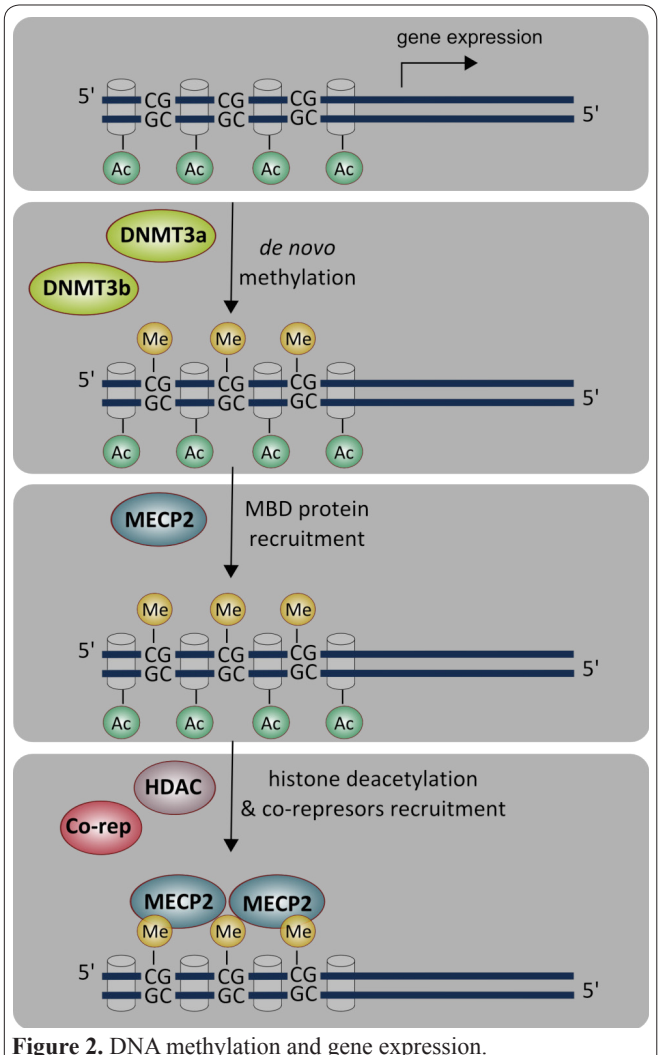

Figure 2. DNA methylation and gene expression.

Table 1. Some hypermethylated and hypomethylated genes and microRNAs in colorectal cancer.

Function	**Hypermethylated in CRC**
WNT/β-catenin signaling	*APC, SFRP1, SFRP2, SFRP4, SFRP5, SOX17, WNT5a, DKK1, DKK3, WIF1, AXIN2*
Cell-cell interactions	*TIMP3, VIM, SEPT9, CDH1, CDH13, ITGA4, ADAM23, RECK, TFPI2, ARHGAP28, PSD, EPHB2*
Cell growth	*IGF2, IGFBP3, NGFR, ESR1, MINT1, MINT2, MINT31, ER, IGFBP3, IGFBP7, SMAD2, SMAD4*
Apoptosis	*RASSF1A, HIC1, DFNA5, RASSF2A, RASSF5 (NORE1), BNIP3, DAPK1, HRK*
Differentiation	*CRABP1, RUNX3, ALX4, GATA4, GATA5, CDX1, FOXL2, ALX4, NEUROG1*
Immune response	*CXCL12, IRF8, PIK3CG, SOCS1*
DNA repair	*MLH1, MGMT, WRN, CHFR*
Cell cycle	*p16/INK4A, KLF4*
Transmembrane transport of ions	*CACNA1G, SLC5A8*
SWI/SNF chromatin remodeling	*HLTF,*
Others	*TMEFF2, UNC5C, DCC, DLEC1, NEURL*
Function	**Hypomethylated in CRC**
Retrotransposons	*LINE-1*
Cell growth	*IGF2*
Function	**miRNAs dysregulated in CRC**
TP53 signaling	*miR-34*
Cell-cell adhesion	*miR-9, miR-34,miR-200*

tumor has metastasized (stage IV) the 5-year survival is around 10%. Therefore, it is important to identify a subset of stage I-III CRCs that can quickly induce metastases. In such cases, anti-metastatic therapy should be included as soon as possible.

Epigenetic modifications frequently go before changes in DNA sequence so they may be associated with early stages of cancer transformation (17). In general, different genes can attribute to cancer initiation, promotion and progression and due to the cancer stem cell theory, there can be specialized cancer stem cells, responsible for cancer invasion and metastasis (18, 19). Each cancer transformation stage has its own "early phase". There are reports indicating epigenetic changes in genes involved in cancer progression (20). Many genes were identified as hyper- and hypomethylated in CRC (Table 1). Identification of epigenetic pattern associated with tumor aggressiveness may help to make a decision on including an anti-metastatic therapy just after CRC diagnosis. Moreover, determining the role of epigenetic changes important for CRC metastasis may help to design epigenetically-oriented drugs. Today, several such drugs are already approved by the FDA and the EMEA for cancer treatment and several others are in clinical development (21).

Two DNMT inhibitors–azacytidine (Vidaza, Celgene) and decitabine (5-aza-2'-deoxycytidine) (Dacogen, SuperGen) – have already been approved by FDA for the treatment of patients with hematological malignancies (22). Since these two agents are currently in phase I clinical trials in patients with solid tumors, therapeutic use of DNMT inhibitors can become beneficial in CRC (23). Zebularine and 5-aza-2'-deoxycytidine-containing dinucleotide (S110), both DNMT inhibitors, are potent candidates for future clinical trials (24). Several DNMT inhibitors are tested in clinical trials in solid tumors (25). Studies conducted on the human colorectal cancer cell lines and mouse models of intestinal tumors demonstrate that DNMT inhibitors suppress intestinal carcinogenesis (26-30). Since overexpression of DNMT3B1 promoted colorectal carcinogenesis *in vivo*, DNMT inhibitors can potentially reverse that effect (28).

The regulation of gene expression through the cytosine methylation acts in concert with the histone modification (31). Therefore, a cross-talk between DNMT and HDAC can occur (32). Thus, it seems reasonable to use demethylating agents in combination with HDAC inhibitors and chemotherapeutic agents. Several studies were conducted on cancer cell lines, including colon cancer cell model (33). Strong cytostatic and apoptotic effects of combined application of DNMT and HDAC inhibitors suggests it may be highly efficient in patients (34, 35). Given that HDAC inhibitors potentiate the effect of standard chemotherapeutics against CRC, the combined action of DNMT and HDAC inhibitors can be even more profitable (36). Phase III trial demonstrated that the combination of hydralazine-valproate (TRANSKRIP™) evoked DNA demethylation and HDAC inhibition in solid tumors (37). Other combination of DNMT and HDAC inhibitors, namely 5-azacytidine and valproic acid, was successfully tested phase I clinical trial in patients with advanced solid tumors, including CRC (38).

Colorectal cancer

Colorectal cancer belongs to the most frequently occurring cancers, both in women and men, in many countries over the world. The induction and development of CRC are determined by genetic and environmental factors. These two classes of risk factors reflect the etiopathogenesis of CRC, which is classified as familial or sporadic. The familial mode of CRC can be attributed to about 30% of all cases, which are further subdivided depending on the presence of the colonic

polyps. The diseases with polyposis include familial adenomatus polyps (FAP) and the hamartomatous polyposis syndromes, e.g., Peutz-Jeghers, juvenile polyposis (39), while those without polyposis include hereditary non-polyposis colorectal cancer (HNPCC, Lynch syndrome I), and the cancer family syndrome (Lynch syndrome II) (40). Approximately 3-4% of colorectal cancer cases are attributed to HNPCC, they can occur at different age and slight variation among patients is likely due to geography and ethnicity. Nearly 1% of CRC cases are due to FAP. Sporadic CRC progresses independently of family history and accounts for about 70% of cases, usually in patients above 50 years old.

Fig. 3 shows colorectal cancer transformation and involvement of genes which are mutated or have altered DNA methylation pattern. The set of genes is chosen arbitrary and some of them may overlap being both mutated and methylated. "Methylation" here is mostly represented by hypermethylation of the CpG dinucleotides in promoters of these genes.

CRC tumorigenesis can be related, as many other cancers, to the alteration in tumor suppressor genes, oncogenes and mutator genes (41). These alternations can occur in germline cells and they are passed to progeny, resulting in familial syndromes. Mutations in somatic cells underline sporadic CRC. Sporadic colorectal carcinogenesis is a multistage process of accumulating alterations resulting in the adenoma-carcinoma transition (42). Aberrant epigenetic regulation of gene expression, methylation of the CpG sites in the promoter sequence, posttranslational modification of histones, chromatin remodeling can be included in these alterations (10, 12, 43-45) (Table 1, Fig. 3). As mentioned, DNA methyltransferases can convert cytosine to 5-methylocytosine at the CpG sites, which is a hallmark of gene silencing. On the other hand, loss of the CpG methylation is associated with gene expression or overexpression. Histone posttranslational modifications such as acetylation and methylation at specific amino acid residues, including lysine and arginine, influence chromosome condensation. Generally, histone hypoacetylation and hypermethylation are markers of transcriptionally inactive chromatin. Also, chromatin remodeling through positioning of nucleosomes at the transcription start site may contribute to gene silencing. These epigenetic modifications can play an important role in the induction and development of CRC (46).

Methylation of DNA can play a special role in CRC pathogenesis in a subset of all cases, called the CpG island methylator phenotype (CIMP) (47, 48). CIMP cases are characterized by a high ratio of methylation of promoters of certain genes and at present there is not a general agreement on the identity of these genes. Most commonly the *CDNK2 (p16/INK4A), hMLH1, MINT1, MINT2* and *MINT31* are considered. However, several other genes can be taken into account, but on the other hand, the *hMLH1* gene seems to be of a special significance, as based on its methylation status, CIMP CRC cases can be categorized into two subgroups determined by its methylation and microsatellite instability (MSI) status (49).

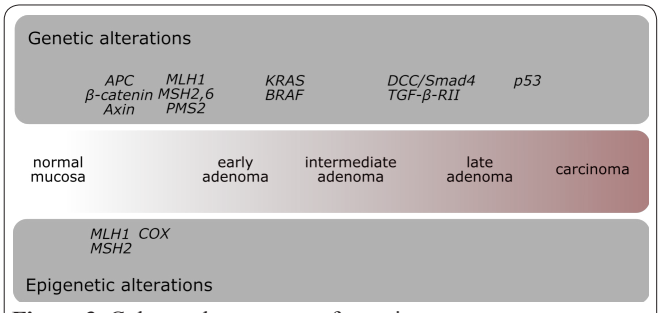

Figure 3. Colorectal cancer transformation.

Metastatic and non-metastatic colorectal cancer

Primary tumors are designated as non-metastatic and are associated with a favorable prognosis, whereas tumors metastasizing to distant organs are associated with an unfavorable prognosis and are called metastatic cancers. Despite the recognition that metastasis is a major cause of death of patients suffering from CRC, the inability to predict the probability, time and location of metastasis hamper the progress in curing this kind of the disease. As mentioned, a subpopulation of cancer cells is considered to be responsible for tumor development, growth and metastasis (50). These cells were denominated cancer stem cells (CSC) as they share common features with normal stem cells. These cells can further invade the organism by dissemination and there were two proposed metastatic model: serial and parallel. The former assumes that metastasis is the final stage of cancer transformation. However, in some patients metastates show no clonal similarity to the primary tumor. In consequence, the latter model proposes that cancer cells disseminate already from the early epithelial alterations. This could explain the cases in which cancer occurrence happened years after the resection of primary cancer. The association between primary tumors and the preferred organ location for secondary metastases gave rise to other two hypothesis. First, the location of metastasis is determined by transfer of cancer cells with blood flow and the other, which is based on ligand-receptor binding at specific locations.

The metastasis is inherent with alterations in DNA, including genetic, epigenetic and cytogenetic changes. Mutations affect genes encoding adhesion molecules (E-cadherin, CD44), proteins inducing cell migration (Met, S100A4, MMPs, uPA) or angiogenesis promoting factors (VEGF) (51, 52). Point mutations were found in the liver CRC metastases in the 12 and 13 codon of the *KRAS* gene. The CRC metastatic cases with KRAS mutations were associated with shorter survival than those without mutation (53).

Using metastatic SW620 and non-metastatic SW480 cell lines derived from one individual as a model system it was found that a large set of genes in these cells were deregulated (54). The peroxiredoxin 3 (Prx3), Trefoil factor 3 (TFF3), Transmembrane 4 superfamily member 1 (TM4SF1), TATA box binding protein (TBP)-associated factor (TAF2N) showed the highest difference in their expression pattern. Prx3 regulates cell proliferation, differentiation and apoptosis (55). Overexpression of TFF3 is associated with aggressive phenotype of CRC as this protein is involved in the regulation of cell migration and metastasis (56). TM4SF1 is a direct androgen-regulated target gene, which is a regulator of cell invasion, metastasis and angiogenesis in PC-3 prostate cell line and HeLa cell line and thus its ove-

rexpression is related to an aggressive form of cancer (57). TAF2N undergoes chromosomal translocation and generates a fusion oncogene with FUS or EWSR1. Nerve growth factor receptor (NGFR), Serum amyloid A1 (SSA1), Cyclin D1 (CCND1), Keratin 13 (KRT13), which were reported to be downregulated in CRC, exhibited the greatest fold change. NGFR is a serine-threonine kinase receptor – a death receptor mediating the transmission of signal to initiate apoptosis. Although the expression of SAA1 decreased during carcinogenesis in the studied cell lines, the research examining samples from CRC patients showed an inverse trend of expressing SAA1 (58). Overexpression of CCND1 was observed in a series of tumors including lung, breast, sarcoma, and colon cancer (59).

KRAS overexpression is strongly associated with the poor prognosis and malignant phenotype, including proliferation, invasion and metastases (60). The *KRAS* mutation was accompanied by the methylation of promoter sequence of Ras association domain family 1A (*RASSF1A*). The hypermethylation of *RASSF1A* appears in the late stage of CRC. On the other hand, although another study showed an increasing promoter hypermethylation, a correlation between hypermethylation and metastasis was not found (61). *RASSF1A* is a tumor suppressor gene and its protein is a regulator of cell cycle control, microtubule stabilization, cellular adhesion, mortality and apoptosis (62). The methylation of *p16/INK4A* was observed and was strongly correlated with malignant phenotype in CRC. The loss of expression of *p16/INK4A* correlated with metastasis to lymph nodes, more advanced stages and a shorter survival. It was suggested that concomitant deregulation of both *KRAS* and *p16/INK4A* may lead to a more aggressive phenotype (63). The p16 is an inhibitor of cyclin-dependent kinase 4 (CDK4) and CDK6, and it functions as a tumor suppressor. The promoter methylation of p16 is correlated with the CpG island methylator phenotype (CIMP) (64).

Disturbances in genome stability, especially loss of heterozygosity (LOH), was identified as a marker in colon tumorigenesis. In addition, the genes involved in the carcinogenesis *DCC, Smad2, Smad7* and *Smad4* are located in 18q and the *TP53* gene is located on chromosome 17p. Loss of 18q and 17p are frequently observed in CRC (65). Loss of 18q and contemporary loss of the deleted in colon cancer gene (*DCC*) is connected with poorer survival of patients with II and III stage of CRC in some studies (66) but not in others (67). Moreover, 18q LOH was later found to be a prognostic marker in stage III and not in stage II of CRC.

Key steps in invasion and metastasis are tightly regulated or influenced by the Rho family GTPases, which expression may be associated with alterations in cell adhesion, cell-matrix, cell-cell interactions and actin organization, ultimately leading to the acquisition of an invasive phenotype. Mutations in *RHO* genes are extremely rare in tumors, but their expression and/or activity is frequently altered in a variety of human cancers. There are reports suggesting an influence of the *BRAF* oncogene, a major downstream regulator of BRAF, for the expression of RhoA (Ras homolog gene family, member A), Rac1 (Ras-related C3 botulinum toxin substrate 1) and Cdc42 (cell division cycle 42) and their function in CRC cell migration and invasion pathways induced by mutations in the *KRAS, BRAF* and *HRAS* genes (68).

Studies on differentially expressed genes in human colon cancer tissues, metastases, and normal tissues allowed to identify the metastasis-associated in colon cancer 1 (*MACC1*) gene (69). Based on *MACC1* mRNA expression in not (yet) metastasized primary colon cancers of stages I, II, and III, negative and positive prediction of the development of metachronous distant metastases was correct to 80% and 74%, respectively. The 5-year-survival for subjects suffering from colon cancer was 80% for *MACC1* low expressors, but 15% for individuals who showed high *MACC1* expression in their primary tumors. MACC1 was found to act as an inducer of migration, invasion and proliferation in cell culture, as well as of liver and lung metastases in several xenograft models. Treatment with hepatocyte growth factor (HGF) leads to translocation of MACC1 from the cytoplasm into the nucleus. There, MACC1 binds to the promoter of the receptor tyrosine kinase Met, transcriptionally regulating its expression. MACC1-induced activation of the HGF/Met signaling pathway results in enhanced cell motility, invasion, and metastasis. In CRC, MACC1 can be considered as a predictor of metastasis allowing for early identification of patients with a high risk of developing metastasis, as the expression of MACC1 is stage-dependent (70-72).

The proto-oncogene and tyrosine kinase receptor, MET is expressed mainly on the surface of epithelial cells. In response to binding of the MET ligand, hepatocyte growth factor (HGF), C-terminal tyrosine residues are phosphorylated followed by a cascade of intracellular signals resulting in the activation of MAPK and/or PI3K/Akt pathways. In this way, aberrant activation of MET leads to increased cell proliferation, invasion, and metastasis (73).The *MET* gene was found to be amplified in approximately 10% of CRCs, and amplification is associated with advanced stages and worse prognoses.

Histone deacetylase 1 (HDAC1) and metastasis-associated protein 1 (MTA1) form the nucleosome remodeling and histone deacetylation (NuRD) complex and can possibly play a central role in cancer development. The expression of MTA1 was reported to be correlated with poorer prognosis and its level was considered as a potential prognostic indicator for colon cancer (74).

Wild-type KRAS/BRAF status is required, but not sufficient to confer sensitivity to anti-EGFR therapy in metastatic CRC. Due to this fact several laboratories studied the potential predictive role of other genetic and epigenetic biomarkers. Considering the EGFR signaling cascade, the RASSF1A seems to be crucial for this transduction pathway regulation because it binds RAS in a GTP-dependent manner and mediate its apoptotic effects. This pathway may be also affected by epigenetic regulation of RASSF1A (75, 76).

Since the discovery of miRNAs and identification of their potential as oncogenes and tumor suppressors, they have been extensively studied in CRC and shown to regulate key CRC signaling. Although they can play an important role in all stages of CRC development, they were considered to be mainly involved in cancer progression (reviewed in (77)). In fact, metastasis is considered as the most pronounced process regula-

ted by miRNA (78). Epithelial mesenchymal transition (EMT), essential for CRC invasion and metastasis, is facilitated by the repression of the *CDH1* gene, a tumor suppressor, which is effectively inhibited by the *SNAI1* gene (79). However, SNAI1 can form a feed-forward loop with miR-34a, which is involved in the control of EMT (80, 81). Moreover, the repression of miR-34a can occur by the methylation of CpG island,associated with cancer transformation (82). Therefore, miR-34a is a good target for epigenetic manipulation to prevent or inhibit metastasis. In fact, MRX34, a compound stimulating miR-34 expression, is the first drug of mi-RNA-oriented cancer therapy (83, 84).

Besides miR-34, also miR-9 can influence the *CDH1* gene expression in CRC (85). However, this regulation is exerted probably on translational level (85), but this does not exclude the potential of this miRNA species to regulate CRC progression through its methylation. This is supported by results of several studies showing an association between methylation status of the miR-9 genes and metastasis in various cancers (86).

CDH1 can be also suppressed by *ZEB1/2,* other masters of ETM (87). They can be targeted by the five-member family of mi-RNA, miR-200, containing miR-141, miR-200a-c and miR-429 (88). Transcription of the family was suppressed by the methylation of CpG island in the promoters of the clusters containing genes encoding miR-200 (89, 90). The expression of miR-200c was associated with breaking multidrug resistance in CRC (91). Consequently, the members of the miR-200 family can constitute a target for anti-metastatic therapy in CRC, based on epigenetic manipulation.

Conclusions and perspectives

Progression of CRC to its metastatic form is the greatest threat of this disease and the common direct of its mortality. Therefore, looking for early markers of high metastatic potential and ways of anti-metastatic therapeutic intervention is also a great challenge in cancer research and is fully justified. DNA methylation, an important epigenetic modification, plays an essential role in regulation of expression of human genes. Therefore, as cancer is a "disease of genes", DNA methylation pattern can change along with the progress of cancer transformation. Several aspects of these changes can be taken into account in their analysis. Firstly, the net methylation level should be evaluated, as it is the easiest way for the detection of changes in DNA methylation. Secondly, the position of methylated CpG islands should be determined as not only net promoter hypermethylation can be important for the resulting expression of a gene, but also its profile can play a role, which was shown for several types of cancer (92). Thirdly, DNA methylation pattern can be stage-specific, which is crucial for research aimed at the determination of early markers of apoptosis as well as for anti-metastatic therapy.

It is commonly accepted that genes, which are changed by mutation in cancer, can be divided into two categories: drivers and passengers. The former are causatively involved in cancer transformation and the latter present mutation resulting from general genomic instability provoked by the former. It is not easy to establish a border between these two groups of genes and it is also possible that there is a mutual exchange between the members of both groups in the course of cancer transformation. DNA mutations are primarily responsible for the induction and early stages of cancer. In CRC, its promotion is accompanied by DNA methylation in certain genes. However, usually all genes important for CRC transformation are divided into those with DNA mutations and those aberrantly methylated. However, sometimes mutation in a driver gene is associated with altered methylation pattern of other genes but frequently the nature of such association is not known, as in the case of *BRAF* and *hMLH1*.

If an abnormal methylation pattern can be detected in a certain gene and it can be directly related to metastatic potential, it could be considered for manipulation to decrease this potential. Although this is conceptually clear, it is difficult to build a strategy to reach this aim. Abnormal methylation pattern could result from the action of methylation agent(s), aberrant functioning of DNA methyltransferases, disturbed action of several enzymes involved in active DNA demethylation and others. It is important that some factors can combine and produce methylation pattern, which can be specific to a specific combination of factors involved in epigenetic modifications. However, DNA methylation can be associated with some alterations in chromatin structure, which are essential for gene activation and inactivation. This potentiate the complexity of the problem as changes in chromatin structure can be induced by other than DNA methylation mechanism and can influence the pattern of epigenetic modifications through the change in accessibility of DNA fragments for epigenetic modifiers. Therefore, a precise manipulation of epigenetic pattern in CRC patients is rather limited. Thinking about inactivation of enzymes involved in epigenetic silencing of tumor suppressor genes has little sense, as such operation could not be limited to a particular gene or set of genes. Instead, this pattern can be useful in prognosis of CRC development. Therapeutic intervention targeting epigenetic pattern must be based on general properties of cancer tissue, featured by global hypomethylation and local hypermethylation. However, changing a methylation pattern within a tissue or organ, if not very specific, can result in unwanted effects caused by changes in methylation pattern of genes, which expression should not be changed. In other words, a non-specific targeting epigenetic pattern can result even in development of secondary cancer(s). Another problem is associated with tissue-specific pattern of DNA methylation. Although in the case of CRC, sampling a target organ does not constitute a really serious problem, but the range of modifications should be determined, especially in the context of tumor resection and possibility of finding evidence of a change in DNA methylation pattern in peripheral blood.

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