Editorial

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Epigenetics in cancer: a promising path to follow?

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Last year it was 10 years since the Cold Spring Harbor meeting (December, 2008) officially acknowledged epigenetics as a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence. The Greek prefix epi- ("over, outside of, around") in epigenetics covers features that are "on top of" or "in addition to" conventional genetics. While each cell in the human body is equipped with the same genetic instructions, epigenetic regulations guide cells during differentiation. In other words, for example, liver cells switch on genes needed for metabolism, neurons turn on neurotransmitter genes, cells in the gastrointestinal tract switch on genes that are important for digestion, and so on. This represents incredibly important processes that help our genes work in the right way. Moreover, the unique epigenetic fingerprint is changing not only during normal physiological processes but also in many diseases. The first human disease linked to epigenetics was cancer, when in 1983, Feinberg and Vogelstein found that the ras oncogenes of colorectal cancer cells were substantially hypomethylated compared to the adjacent analogous normal tissues from which the tumors derived [1]. Nowadays, epigenetics represents a very exciting and fast-paced area of research, particularly in cancer as is documented by several articles in the current issue of *Clinical Chemistry and Laboratory Medicine* (*CCLM*) [2–5].

Within cells there are three major mechanisms that can interact with each other in order to silence genes and are considered as epigenetic regulation: DNA methylation, histone modifications and non-coding RNA mechanisms. DNA methylation represents one of the most extensively studied and well-described epigenetic alterations. Our knowledge about it dates back to 1969, when Griffith and Mahler suggested that DNA methylation may be important in long-term memory [6]. This process is catalyzed by DNA methyltransferases (DNMTs); DNMT3A and DNMT3B are responsible for *de novo* DNA methylation and DNMT1 distinguishes hemimethylated DNA, and so has a function of maintenance DNA methyltransferase. DNMTs catalyze the covalent addition of a methyl group to the 5-carbon of the cytosine creating the 5-methylcytosine (5-mC). On the other hand, ten-eleven translocations (TETs) are a family of proteins accountable for converting 5-mC to the 5-hydroxymethylcytosine (5-hmC) which finally leads to demethylation [7]. DNA methylation is present mainly in the context of CpGs dispersed throughout the genome or in DNA repetitive regions. These CpG-rich regions are known as CpG islands. CpG islands are huge sequences (~800–900 nucleotides on average) where is a high presence of CpGs (~10%) and C + G content (>55%) [8]. DNA hypermethylation in promoter regions participates in gene silencing, whereas DNA hypomethylation can activate genes and initiate chromosome instability. In cancer, aberrant DNA hypermethylation is typically observed in the promoter/exon1 regions of various tumor suppressor genes [9], DNA repair genes and cell cycle regulators leading to their transcriptional silence [10, 11].

Besides DNA methylation, histone modifications act as key regulators in the epigenetic control of gene transcription in cancer cells. Histones are small basic proteins, located in the nucleus of eukaryotic cells, that are helpful in forming DNA strands into nucleosomes by creating molecular units around which the DNA is packed. Their main functions are to condense DNA and regulate chromatin. There are many histone modifications, known as "the histone code", that together with DNA methylation regulate the expression of specific genes [12]. The most common alterations that initially affect lysine residues of histone tails include methylation and acetylation. Specifically, histone acetylations are associated with euchromatin and the up-regulation of gene transcription. Acetylations are affected by two classes of enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs). HDACs are often upregulated in cancer cells, leading to differences in expression and activity of selected proteins involved in carcinogenesis [13].

Next widely studied epigenetic regulators are microRNAs (miRNAs). This topic is discussed in detail in the current issue of *CCLM* in the review article by Terrinoni et al. [3]. MiRNAs represent a group of small, endogenous, ~22 bp long non-coding RNAs that are part of gene expression regulatory network in almost all crucial cellular process, such as the regulation of cell proliferation, differentiation and apoptosis. Studies show that miRNAs influence the development of many diseases, particularly cancer [14, 15]. Human genome encodes approximately

2700 mature miRNAs that may regulate human transcripts. These non-coding RNAs represent the negative regulators of gene expression at the post-transcriptional level. As most target sites on mRNA have only intermediate base complementarity with their matching miRNAs, individual miRNAs may interact with several diverse mRNAs and in this way inhibit their translation to polypeptides. MiRNAs hold the promise of being ideal biomarker molecules for healthcare needs, particularly in cancer, but this field is still in its beginning and far from the use in practice. Non-coding RNA mechanisms are represented not only by miRNAs; long non-coding RNAs (lncRNAs) are also involved in epigenetic regulation. Articles by Zong et al. and Liu et al. in the current issue of *CCLM* discuss the role of lncRNAs in the diagnosis and prognosis of gastric and colorectal cancer [4, 5].

Changes in epigenome can be detected using plenty of techniques which are described in detail in several reviews [16–18]. Briefly, the most common techniques for DNA methylation analysis are based on bisulfite conversion. During bisulfite modification unmethylated cytosines are transformed to uracils, which are then transformed to thymines within DNA amplification by PCR, while methylated cytosines are protected from bisulfite modification. These changes in the DNA sequence can be subsequently determined by methylation specific PCR (MSP) or by DNA sequencing. For monitoring of posttranscription changes, such as miRNAs expression, methods based on real-time PCR are usually used. On the other hand, thanks to mass spectrometry we are able to analyze posttranslational protein modifications. Every type of posttranslational alteration adds a different mass to the studied molecule and due to the high resolution of modern mass spectrometers mainly when "soft" ionization techniques are used, the investigation of posttranslational modifications has been significantly simplified [16]. For monitoring of changes in chromatin structure immunoprecipitation (ChIP) is used which tracks DNA-protein interactions. And finally, analysis of epigenetic regulating enzymes is based on the analysis of alterations in mRNA and protein levels, which can be performed using real-time PCR, respectively, Western blot techniques. Nowadays, rapidly developing technologies provide us new possibilities in the mapping of epigenetic alterations in a large range. Most recently, microarrays and ultra-high throughput technologies using massive parallel sequencing have given us new exciting tools for epigenomic investigation, but they also represent challenges in data processing, statistical analysis and biological interpretation of observed differences.

Epigenetics has many potential medical applications in the field of cancer. It is evident that epigenetic modifications represent an interesting area for biomarker discovery [19–22]. Nowadays, several epigenetically modified tumor-associated nucleic acids have been found in the plasma/serum of cancer patients and detection of circulating epigenetic markers provide us with new possibilities in cancer detection and treatment management [23]. In addition to plasma/serum, epigenetic markers may be detected in other bodily fluids, such as urine, sputum and breast ductal lavage [24]. Moreover, several studies have discussed the importance of epigenetics in the early stages of tumors setting them as the ideal marker for screening.

We can define several types of *biomarkers* – detection and diagnostic ones, prognostic and predictive ones or biomarkers used for disease monitoring. From the viewpoint of predictive biomarkers, in glioblastoma, epigenetic downregulation of the *MGMT* (O6-methylguanine-DNA methyltransferase) gene by its promoter methylation is definitely the epigenetic fingerprint with the highest implementation in clinical practice. The *MGMT* gene encodes a DNA repair enzyme that removes alkyl adducts from the O^6-position of guanine and so prevents errors during DNA replication and transcription. On the other hand, it also protects tumor cells from the chemotherapy effects of alkylating agents such as temozolomide. A number of studies have shown that *MGMT* promoter methylation makes tumor cells more sensitive to alkylating drugs and results in a better response and longer survival in glioblastoma patients [25].

The Septin 9 (*SEPT9*) gene belongs to the group of methylation-based biomarkers for early detection of cancer. The assay Epi proColon was approved for clinical use in April 2016 by the US Food and Drug Administration (FDA) as a screening test for colon cancer. This assay is based on detection of the methylated *SEPT9* and has been included in screening programs [26]. Methylation of other candidate genes *SHOX2* and *GSTP1* are investigated for possible use as diagnostic/screening biomarkers. DNA methylation of the *SHOX2* (short stature homeobox 2) gene is now recommended in the diagnostic/detection of malignant lung disease particularly in patients where histology and cytology results are unclear [27]. And finally, methylation in *GSTP1* represents a potential biomarker in prostate cancer screening [28]. In the field of urology, the promising project UroMark is now running – a urinary biomarker assay for the detection of bladder cancer. This study is focused on developing a targeted bisulfite next-generation sequencing panel, which could help us to find bladder cancer in urinary sediment DNA with high sensitivity and specificity [29].

The next challenge in cancer epigenetics is in the area of treatment where new therapeutic approaches are

needed urgently. Epigenetic alterations can be reversed more easily than mutations affecting the genetic code therefore epigenetics propose a promising and valuable approach of therapy. In hematological cancers, such as myelodysplastic syndrome (MDS), drugs that reverse epigenetic alterations are commonly used. Nowadays six drugs influencing the epigenome have been approved by the FDA for cancer treatment and many more candidates are under vigorous investigation [30]. They can be generally classified as DNA methyltransferase inhibitors (DNMTi) and histone deacetylases inhibitors (HDACi). Two inhibitors of DNA methyltransferases, azacytidine (Vidaza®) and decitabine (Dacogen®) are a part of the standard therapy of patients with MDS. Also, a growing list of broad spectrum HDACi can be seen; namely vorinostat (Zolinza®), romidepsin (Isotadax®) and belinostat (Beleodaq®) are used in treatment of cutaneous T-cell lymphoma, and panobinostat (Farydak®) is used for drug-resistant multiple myeloma [30]. It is evident that hypomethylating agents and HDACi that reverse cancer-associated histone modifications have significantly increased our arsenal of cancer drugs particularly for hematological malignancies. From the viewpoint of post-transcriptional regulation, several miRNA-based therapeutics are in clinical testing including a mimic of the tumor suppressor miR-34, which has achieved phase I clinical trials for cancer treatment [31]. However, in epigenetic field and current treatment strategies, there are still many questions to be answered before we can use our basic knowledge in the clinical area, mainly in solid tumors. The most important issue of the mentioned strategies is the target selectivity and the fact that not all cancers are equally susceptible to "epigenetic therapies". On the other hand, during the last few years DNA methylation editing techniques have been designed by fusion of inactivated Cas9 with the DNA methylation/demethylation enzymes DNMT/TET (dCas9- DNMT/TET), enabling *in vitro* and *in vivo* targeted rearrangement of DNA methylation in the mammalian genome [32–34].

One of the most alarming issues in cancer therapy is drug resistance. Current research is significantly focused on the search of mechanisms which could elucidate this phenomenon that could help us to overcome chemoresistance. A lot of genetic abnormalities are connected with this process, for example, aberrations in genes involved in DNA repair, drug uptake, apoptosis and cell cycle regulation. Recently, increasing interest has been given to epigenetic mechanisms in drug resistance. The most obvious example is platinum-based chemotherapy. Cisplatin can generate methylation in the DNA mismatch repair gene (*MLH1*) [35]. This well-known epigenetic event probably represents a major molecular aberration in the development of acquired resistance to platinum-based chemotherapy in ovarian cancer patients [36]. Hence, hypermethylation represents a fascinating target for influencing the tumor biology and potentially the prediction, prevention or overcoming therapy resistance. Another epigenetic example of drug resistance is mentioned in the current issue of *CCLM* by Kuhlmann et al., this group analyzed the profile of extracellular vesicle-associated miRNAs in platinum-resistant ovarian cancer patients [2].

And finally, we should keep in mind the importance of epigenetics in cancer prevention. It is evident that prevention is better than treatment and epigenetics has a great potential in this field. Many dietary components and a healthy lifestyle show anticancer properties and may be important in cancer prevention. Dietary agents including fruits, vegetables and spices have the potential to epigenetically regulate gene expression; and are important mainly in the deregulation of cancer-related genes, such as tumor suppressor genes, cell cycle regulators and the genes involved in apoptosis [37, 38]. Several nutrients in the diet such as folic acid, vitamins B6 and B12 have key roles in the methylation of biological substrates and can influence DNA methylation either by changing the availability of methyl donors or by modulation of the DNMTs' activity. Moreover, microorganisms in the gastrointestinal tract produce low molecular weight bioactive substances such as folate, butyrate, biotin and acetate that may influence epigenetic processes [39]. Remarkably, some studies suggest that the maternal diet, alcohol consumption and smoking may influence cancer incidence in the offspring indicating possible transgenerational effects of epigenetic diet on cancer prevention [40].

Although aberrant epigenetic patterns in cancer were first reported more than three decades ago, we have still plenty of questions regarding epigenetics in cancer development and progression. The clinical significance of epigenetic biomarkers may play an important role in personalized medicine in cancer patients, but a number clinical trials must be performed to elucidate the real significance of these biomarkers. In conclusion, it is evident that the roads leading to effective cancer prevention, screening or therapies are long and we do not have a complete map that would lead us to success. However, epigenetics at least gives us a promising path to follow.

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