Deregulation of epigenetic mechanisms in cancer

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Abbreviations: AML – acute myeloid leukemia; GBM – glioblastoma; HAT – histone acetyltransferase; HDAC – histone deacetylase; HMT – histone methyltransferase; IDH – isocitrate dehydrogenase; PRC – Polycomb Repressive Complex; TCGA – The Cancer Genome Atlas

ABSTRACT

Gene expression of both normal and cancer cell is tightly regulated by specific transcription regulators and epigenetic mechanisms such as DNA methylation, histone modifications (acetylation, methylation, phosphorylation), nucleosome remodeling and non-coding RNAs. Deregulation of epigenetic mechanisms plays a pivotal role in cancer, although researchers debate if it is a cause or a consequence of oncogenic transformation. Independently from the way in which epigenetic alterations arise in cancer, downstream effects will result in profound changes in transcriptomic and subsequently proteomic profiles. In most cases, changes in expression of epigenetic genes produce functional advantages in cell proliferation, tumor growth and/or migration capacity. Most of epigenetic changes in cancer are triggered by genomic alterations in specific genes that are involved in controlling one of the epigenetic mechanisms. However, there are also mutations in cell metabolism-related genes that affect activities of DNA demethylating enzymes and histone modifiers. Histone modifications are deregulated in cancer mostly due to alterations in genes coding for enzymes that attach or remove histone modifications. Mutations in genes coding for nucleosome remodelers result in aberrant global chromatin organization and facilitate subsequent global alterations of gene copy number or translocations. Recent advancements in next generation sequencing allowed for more precise mapping of global changes in the epigenetic landscape in cancer.

A BRIEF OVERVIEW OF EPIGENETIC MECHANISMS

Despite having the complete genetic information in each cell, each cell type displays its own characteristic transcriptome defined by a set of active genes, whereas those genes specific for other cell identities are silenced. One of the important mechanisms of differential reading of the genetic information is based on epigenetic mechanisms. Epigenetics has been defined as *''the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence*". Epigenetic processes are based on reversible marks and modifications of chromatin, a macromolecular structure consisting of unmodified and chemically modified nucleic and amino acids found in the nucleus. Chromatin consists of approximately 3 meters of DNA which is tightly packed by wrapping a DNA chain around a nucleosome, a protein complex consisting of two of the histones H2A, H2B, H3, and H4. This "beads-on-a-string" structure coils into a 30 nm diameter helical structure known as the 30 nm fibre and then compacts further to higher order chromatin structure. The spatial arrangement of the chromatin within the nucleus is not random. Compacting chromatin at a particular gene is crucial for its expression as its defines accessibility of DNA for transcriptional regulators and affects further cell-fate decision making processes in both normal cell differentiation and oncogenic transformation. Remodeling of chromatin can be achieved in several, interconnected ways. The most well described epigenetic mechanisms, which mediate changes in gene function, are: DNA methylation, histone modifications (acetylation, methylation, phosphorylation), nucleosome remodeling and non-coding RNAs. However, fine tuning of chromatin structure can be also effected by replacement of histone variants and positioning of the nucleosome, both processes known to regulate access of transcription factors to their target DNA and influence chromatin state and transcription.

Deregulation of epigenetic mechanisms plays a pivotal role in cancer, although researchers debate if it is a cause or a consequence of oncogenic transformation. Independently from the way in which epigenetic alterations arise in cancer, downstream effects will result in profound changes in transcriptomic and subsequently proteomic profiles. Oncogenic transformation may deeply alter the epigenetic information encoded in the pattern of DNA methylation or histone modifications [1,2]. Deregulation of the epigenetic landscape can also result from activation/inactivation of the enzymes that

maintain and modify the epigenome [3]. Here we discuss the epigenetic mechanisms involved in cancer development and progression. Most of the epigenetic mechanisms involved in tumorigenesis have been discovered in two types of cancer: glioma and leukemia, therefore we will confine our discourse to the examples described in these two malignancies.

DNA METHYLATION

DNA methylation is a process by which methyl groups are added to the DNA molecule. In eukaryotes, methylation occurs at the cytosine residues of DNA. In plants, DNA methylation is found in three different sequence contexts: CG (or CpG), CHG or CHH (where H represents A, T or C). In mammals, DNA methylation is almost exclusively found within CpG dinucleotides, and is strongly correlated with transcriptional repression [4]. Regions of stably silenced chromatin such as centromeric, pericentromeric and repetitive element containing regions are heavily methylated. In contrast, so called CpG islands, which means large stretches of CpG-rich sequences that form regulatory units located mostly in gene promoters and regulatory sequences, are largely unmethylated (<10%). This basic bimodal methylation pattern is maintained through every cell division and serves as a global repression mechanism. Changes in DNA methylation are introduced through targeted *de novo* methylation and demethylation [5]. Gross changes in DNA methylation are essential for normal development and are associated with a number of key processes including genomic imprinting, X-chromosome inactivation, repression of transposable elements, aging and carcinogenesis.

HISTONE MODIFICATIONS

In addition to its role as a DNA packaging device, a nucleosome is a signaling module through which changes in environmental and metabolic conditions can influence genomic functions [6]. Histone proteins can be affected by a variety of posttranslational modifications (histone marks), which are critical to dynamic modulation of chromatin structure and function, as well as to transcription regulation. At least nine different types of histone modifications have been described, the best understood being lysine acetylation, lysine and arginine methylation, serine/threonine/tyrosine phosphorylation, and serine/threonine ubiquitylation [7]. Post-translational modifications of histones regulate all processes involving DNA as a template, including replication, transcription and repair. Numerous covalent histone modifications have been described at active or inactive loci. For example, acetylation of N-terminal lysine residues of histones H3 and H4 is typically associated with the active chromatin, while methylation of lysines 9 and 27 of histone H3 are the hallmarks of condensed chromatin at silent loci [8].

Most described histone modifications lie within the N-terminal histone tails, which protrude from the nucleosome core. Those modifications may affect electrostatic interactions between the histone tails and DNA to "loosen" a chromatin structure influencing gene expression. Adding acetyl- or phosphor- groups effectively reduce the positive charge of histones, and this has the potential to disrupt electrostatic interactions between histones and DNA [9]. Also single-site modifications may result in gross structural chromatin changes. For instance, acetylation of lysine 16 on histone H4 inhibits the formation of higher order chromatin structure and functional interactions between a non-histone protein and the chromatin fiber or phosphorylation of serine 10 on histone H3, which occurs genome-wide during mitosis and is associated with chromatin condensation [10]. Moreover, modifications may affect histone-histone interactions within the nucleosome core. For example, acetylation of lysine 64 on histone H3 is enriched at the transcription start sites of active genes, where it regulates nucleosome stability and facilitates nucleosome eviction and hence gene expression [10,11].

A higher level of complexity arises from collaborative or antagonistic cross-talks between different epigenetic modifications. It is now well established that there is an intense cross-talk between them, which can occur on the same histone (*cross-talk in cis* e.g. lysine 9 on histone H3 can be acetylated or methylated having an opposing effect on transcriptional regulation), between different histones within the same nucleosome (*cross-talk in trans* e.g. methylation of lysine 4 and lysine 79 on histone H3 is totally dependent upon ubiquitination of lysine 123 on histone H2B), or across different nucleosomes (*nucleosome cross-talk*). Furthermore, binding of a protein to a particular modification can be disrupted by an adjacent modification. For example, Heterochromatin Protein 1 (HP1) binds to chromatin with di- or trimethylated lysine 9 on histone H3, but during mitosis this binding is disrupted due to phosphorylation of serine 10 on histone H3 (for a review see [9]).

Combinations of different histone modifications create a pattern known as "*a histone code*" and form epitopes to recruit other proteins. Current evidence indicates that these modifications recruit transcription factors, chromatin remodelers or chromatin structural proteins that are involved in chromatin condensation or de-condensation and contribute to the formation and maintenance of active or repressive chromatin states [12]. Numerous chromatin-associated factors can specifically interact with modified histones via many distinct domains such as: bromodomain which predominantly recognizes acetylated residues, chromodomain, MBT domain and Tudor domain, which recognize methylated histones or plant homeodomain (PHD) zinc fingers, which recognize and bind both acetylated or methylated residues [12].

NUCLEOSOME REMODELING

Transcription sites of genes that are tightly regulated in time or/and in space, such as genes related to embryonic development or genes specific for highly specialized cells i.e. neurons, are repressed in most cells. Tight nucleosome organization at regulatory regions of those genes blocks access to transcription regulators and make them effectively repressed. Nucleosome remodeling at

the repressed genes is driven by chromatin remodeling complexes (CRCs) which utilize the energy of ATP to disrupt nucleosome - DNA contacts, move nucleosomes along DNA, and remove or exchange nucleosomes. The most important nucleosome remodeling proteins are ATP-dependent SWI/SNF family members which have been discovered in yeasts (SWI or SNF – switching defective or sucrose non-fermenting) but are evolutionarily conserved, and homologous proteins were subsequently identified in flies, plants, and mammals. These are ATP-binding helicases which share affinity for the nucleosome and display DNA- and nucleosome-dependent ATPase activity *in vitro*. In humans the complex contains two mutually-exclusive ATPase enzymatic subunits BRM (*Brahma*, SMARCA2) and BRG1 (*Brahma related gene 1,* SMARCA4), and 10 or more BRM/BRG1-asssociated factor (BAF) subunits. SWI/SNF proteins form complexes with the accessory subunits containing interaction domains that may directly regulate the enzymatic activity of the entire complex, facilitate binding to transcription factors and other chromatin modifying enzymes, and target the complex to DNA and modified histones. These complexes generally do not bind at promoters, which are less compact than other genomic regions, but interact with Actin and Actin-related proteins shortly called Arps. One possible role of Actin in these complexes is to act as an exchange factor to remove ADP from the active site and promote a conformational twist of the Brg/Brm ATPase [13].

DEREGULATION OF EPIGENETIC MECHANISMS IN CANCER

DEREGULATION OF EPIGENETIC ENZYMES

Although post-translational modifications of histones are widespread and diverse, they are spatially and temporally regulated in a highly intricate manner. Cumulative evidence shows that epigenetic patterns are distorted in malignancy on a genome-wide and a particular gene loci level. Those distorted patterns often reflect the altered expression of the enzymes that control histone modifications. Similarly, gene expression patterns are deregulated and show correlation with altered histone modifications at both the candidate loci and genome-wide levels. Deregulation of the epigenetic landscape can occur due to aberrant activation or inactivation of the enzymes that maintain and modify the epigenome. If you consider patterns of histone marks as "an epigenetic code", all proteins regulating histone modifications and functions could be divided into three major classes: chromatin writers (acetylases, metyltrasferases, kinases), erasers (deacetylases, demetyltrasferases, phosphatases) and readers (modified histones recognizing proteins, chromatin remodelers). Chromatin writers and erasers are opposing enzymes, which introduce or remove histone modifications, respectively. Comprehensive molecular characterization of gliomas and leukemias performed by The Cancer Genome Atlas (TCGA) consortium revealed importance of epigenetic alterations in these tumors. Discovery of which epigenetic enzymes are deregulated in cancer provides a rationale for targeting specific enzymes with the aberrant expression/activity in cancer cells. Studies carried out by our group revealed the cytotoxic effects of inhibitors targeting major classes of epigenetic enzymes in C6 rat glioma cells [13].

DEREGULATION OF EPIGENETIC ENZYMES EXPRESSION

Acetylation of lysines in histones is regulated by histone acetyltransferases (HATs) and deacetylases (HDACs). HDACs remove the acetyl groups of lysine residues of histone tails, which leads to local chromatin compaction and transcriptional repression. HATs and HDACs have relatively low substrate specificity and cooperate with other family members forming multi-subunit protein complexes, which determines their specificity [14]. Their expression or enzymatic activities are altered in numerous malignancies and contribute to malignant transformation. For example, the known pathogenic event in acute promyelocytic leukemia is fusion of the promyelocytic leukaemia (PML) and the promyelocytic leukaemia zinc-finger (PLZF) proteins to retinoic acid receptor-α (RARα). PML–RARα and PLZF–RARα are known to recruit HDACs to mediate aberrant gene silencing, which contributes to disease pathology [15]. HDACs can also interact with other proteins than histones, i.e. oncogenic proteins BCL6 recruit and are regulated by dynamic acetylation by HDAC4, -5, or –7 [16].

Our group determined the expression profile of *HDAC1-11* in gliomas of different malignancy grades within the public TCGA (The Cancer Genome Atlas) database. The expression of most HDACs correlated inversely with malignancy grade, which means that their expression was lower in malignant gliomas. Only *HDAC1,2,3* and *HDAC7* levels were up-regulated in malignant gliomas (Waś *et al*., submitted). Another transcriptomic studies also suggested inverse correlation with tumor grade of 7 out of 8 HDACs. The levels of acetylated histone H3 (but not histone H4) was higher in glioblastomas (WHO grade IV tumors) than normal brains [17]. We performed knockdown of HDAC1 or 2 in human glioblastoma cells and demonstrated significant decreases of cell proliferation. Moreover, many HDAC inhibitors show anti-proliferative effects when tested in different glioma cells (Waś *et al.*, submitted).

Histone methyltransferases (HMTs) catalyze the transfer of methyl groups from S-adenosylmethionine to lysine and arginine residues of histone proteins, mostly within the N-terminal tails. The best-characterized sites of histone methylation occur on lysine and arginine residues, which could be mono-, di- or tri-methylated. HMTs tend to be relatively specific enzymes. Furthermore, those enzymes modify the appropriate lysine to a certain degree: i.e. SUV39H can only di- and tri-methylate lysine 9 on histone H3, and other enzyme G9a can carry mono- and dimethylation of lysine 9 on histone H3 [18]. Histone methylation is reversed by histone demethylases. They are also sensitive to a degree of lysine methylation. While most of lysine residues are dynamically methylated and unmethylated by a set of opposing enzymes, arginine

methylation is a relatively stable mark, and it is unclear if the modification can be enzymatically reversed [19-21].

Increasing evidence supports involvement of histone methyltransferase EZH2 (Enhancer of zeste homolog 2) in regulation of cell proliferation, apoptosis, invasion and angiogenesis *in vitro* [22-26] and *in vivo* [26,27]. EZH2 is the catalytic subunit of Polycomb repressive complex 2 (PRC2), which targets lysine 27 of histone H3. Methylation of lysine 27 on histone H3 (H3K27me3) is commonly associated with silencing of differentiation genes in organisms from plants to flies to humans. Promoters of some genes implicated in tumorigenesis, including *c-MYC, BMPR1b* or *mir 200/a/429*, are direct targets of EZH2 and subjects of H3K27me3 modifications [28-30]. Increased immunostaining for EZH2 was significantly correlated with WHO tumor grade and worse prognosis in gliomas [31]. Growing evidence emphasizes also the role of EZH2 in the maintenance and renewal of cancer stem cells in glioblastoma.

The expression of histone lysine methyltransferases SUV39H1 and SETDB1 is up-regulated in cultured glioma cells and in glioma tissues compared to normal brains, which correlates with tumor malignancy. Treatments that altered SUV39H1 and SETDB1 expression affected proliferative and apoptotic potential as well as migratory and colony formation capacity of glioma cells [32]. Recently our group demonstrated binding of histone lysine methyltransferase G9a to the promoters of autophagy (*LC3B, WIPI1*) and differentiation-related (*GFAP, TUBB3*) genes in glioma stem cells. Pharmacological inhibition of G9a up-regulated the expression of autophagy and differentiation-related genes and induced differentiation of glioma stem cells in autophagy-dependent manner [33].

The protein arginine methyltransferase (PRMT) 1 and 5 expression is up-regulated in glioma tissues and cell lines compared with normal brains [34,35]. Knock-down of PRMT1 resulted in inhibition of proliferation and induction of apoptosis in four glioma cell lines, as well as decreased tumor growth *in vivo* [34]. Genetic attenuation of PRMT5 led to cell-cycle arrest, apoptosis, and loss of cell migratory activity [36].

The histone lysine demethylases (KDM) 1 and 5B are also overexpressed in gliomas when compared to normal brains. KDM1 preferentially removes methyl groups from the mono- and di-methylated lysine 4 in histone H3. Pharmacological inhibition of KDM1 in patient-derived primary glioblastoma cell cultures decreased cell proliferation, reduced the expression of stemness markers CD133 and NESTIN in glioma cells and significantly diminished tumor growth in a murine xenograft model [37]. Knock-down or overexpression of KDM5B affects glioma growth both *in vitro* and *in vivo* [38].

Histone phosphorylation is much less studied modification than acetylation or methylation, although it plays a crucial role in processes such as DNA repair, mitosis, meiosis, transcription regulation or apoptosis. All four nucleosomal histone tails contain serine, threonine or tyrosine, which can be phosphorylated by a number of protein kinases and dephosphorylated by phosphatases. Characterization of their biological functions in cancer remains open question, however a few recent publication suggest that the expression of enzymes that modify histones by phosphorylation is also deregulated in gliomas. High levels of phosphorylated residues such as threonine 6, serine 10 and tyrosine 41 on histone H3 are signatures associated with a poor prognosis of glioblastoma patients. These signatures correlate with the high activity of the PKC, Aurora-B and JAK2 kinases, respectively and are abolished by specific kinase inhibitors [39].

Numerous proteins act as "readers" of the "epigenetic code" and recognize a modified histone residue, its type (acetylation *vs* methylation) or degree of modification (mono- *vs* trimethylation of lysine) [12]. Chromatin-associated complexes typically contain multiple readers of epigenetic marks, which guarantees a proper response to different signals. Binding of the readers recruits or stabilizes various components of the nuclear machinery at specific genomic sites. Although in recent years there has been increasing interest in developing small molecules that can target chromatin readers in cancer, the data about their deregulated expression in cancers are very limited.

MUTATIONS IN EPIGENETIC ENZYMES

Genes coding for epigenetics enzymes are frequently mutated in cancer [40]. In addition to increased gene expression, also mutations in the *EZH2* gene can affect histone modifications and have recently been reported in prostate [41] and breast [42] cancers, and in several types of leukemia [43,44]. Recurrent mutations have also been detected in genes coding for histone lysine demethylases KDM5A (JARID1A), KDM5C (JARID1C), and KDM6A (UTX). Mutations in *KDM6A*, in particular, are prevalent in a large number of solid and hematological cancers [45].

The exome sequencing of glioblastoma uncovered mutations in many genes involved in epigenetic regulation, including genes coding for methyl-CpG binding domain protein 1 (MBD1), histone deacetylases HDAC2 and HDAC9, histone demethylases JMJD1A and JMJD1B, histone methyltransferases SET7, SETD2, SETD7, MLL3, MLL4. Among those enzymes one of the most interesting is SETD2, the only known histone H3 lysine 36 (H3K36) trimethyltransferase in humans. Tumors with the mutated *SETD2* show a substantial decrease in H3K36me3 levels suggesting that the mutations result in loss-of-function [46].

DNA methylation status is dynamically regulated by DNA methylation and demethylation reactions. There are three active DNA methyltransferases in mammals: DNMT1, DNMT3A, and DNMT3B. DNMT1 maintains DNA methylation at semi-methylated DNA after DNA replication during cell divisions, whereas DNMT3A and DNMT3B are responsible for establishing *de novo* DNA methylation [47]. A third member of the DNMT3 family, DNMT3-like (DNMT3L), which has no catalytic activity, acts as a regulator of DNMT3A and DNMT3B [48, 49]. Mammalian DNMT2 is a tRNA methyltransferase rather

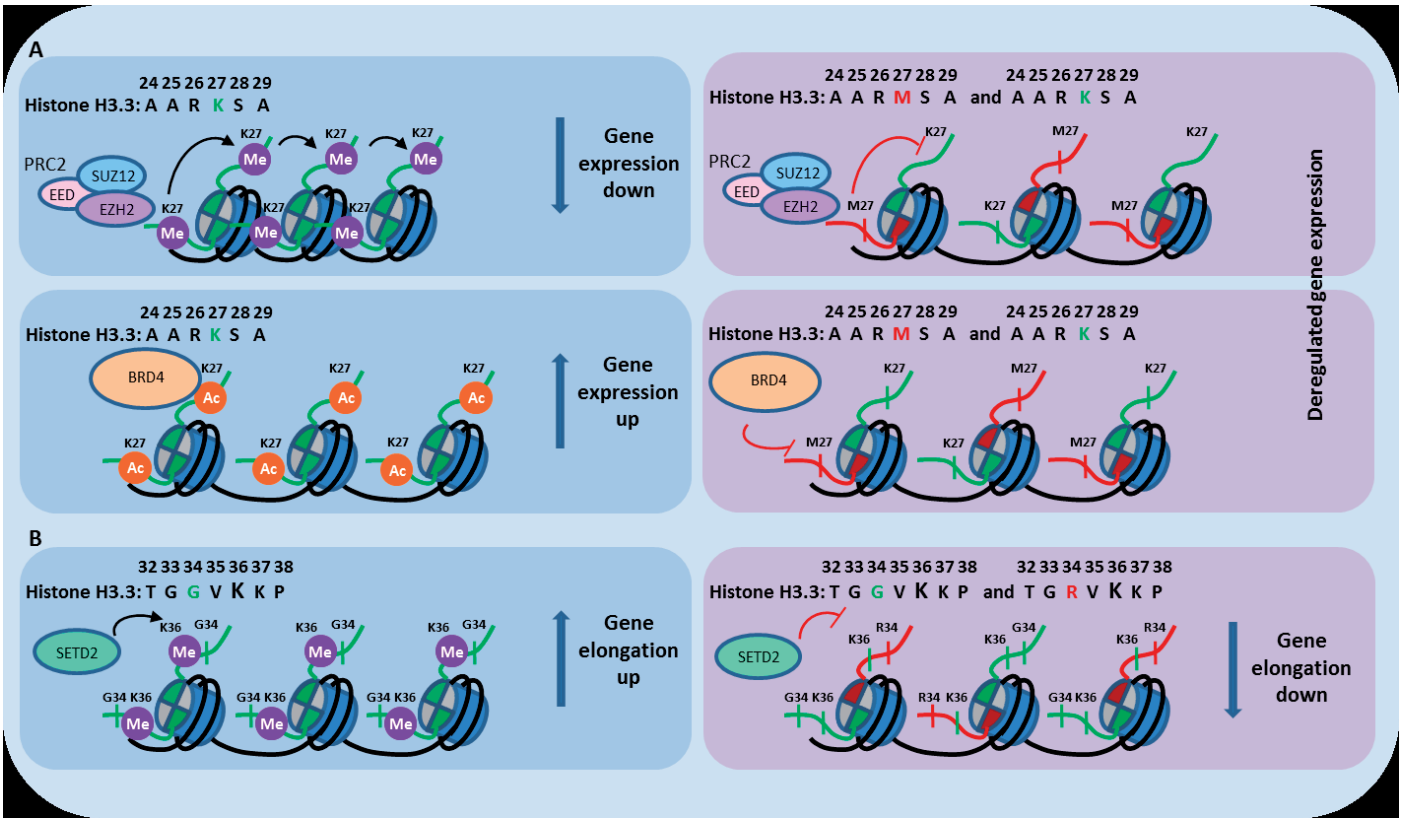

Figure 1. Consequence of histone H3.3 mutations in gliomas. (**A**) H3F3AK27M mutations changing a lysine to methionine at the position 27 of H3.3 alter the ability of this critical residue to be methylated and acetylated, causing aberrant chromatin remodeling, loss of interaction with a repressive PRC2 complex and deregulation of gene expression. (**B**) H3F3AG34R mutations changing a glycine to arginine at the position 34 of H3.3 block the ability of SETD2 histone methyltransferase to methylate H3K36 causing deregulation of gene elongation during transcription

than a DNA methyltransferase and has been renamed tRNA aspartic acid methyltransferase 1 [50]. Mutations in the *DNMT3A* gene have been found first in acute monocytic leukemia [51-53] and subsequently in patients with various other haematological malignancies. TCGA analysis of *de novo* acute myeloid leukemia (AML) patients revealed *DNMT3a* as a third most frequently mutated gene in this malignancy [54]. The R882 hotspot mutation occurs most frequently in AML and acts as a dominant-negative inhibitor of wild-type DNMT3A enzymatic activity. Loss of murine *Dnmt3a* causes human stem cells expansion, clonal dominance, aberrant DNA methylation, and eventually hematological malignancies [55]. Ten-Eleven-Translocation 2 (TET2) is an enzyme which catalyzes the conversion of 5-methylcytosine into 5-hydroxymethylcytosine (5-hmC) and thereby influences the epigenetic state of DNA. Loss-of-function *TET2* mutations were identified in 20–30% myeloid neoplasms [56,57].

TRANSLOCATIONS

The roles of histone methyltransferases in disease pathogenesis have been studied most extensively within the context of leukemias harboring rearrangements in the mixed lineage leukemia (*MLL1*) gene, as this was the first identified cancer-associated mutation of a gene encoding a histone methyltransferase. As a consequence of chromosomal translocations, a fusion oncoprotein is generated, in which the amino-terminal portion of MLL1 is joined in frame to the carboxyl terminal portion of one of over 70 partner genes, the most common of which include *AF4, AF9, AF10, ENL* and *ELL*. This fusion results in the loss of catalytic MLL ability to methylate lysine 4 of histone H3 (H3K4), but via interactions with MLL fusion partners, two nuclear proteins: DOT1L and the super elongation complex (SEC) can be aberrantly recruited to MLL fusion target genes to drive gene expression. Discovery of oncogenic effects exerted by MLL fusions spurred development of inhibitors for therapeutic intervention [58]. Cytogenetic studies, as well as next generation sequencing of various cancer genomes, have demonstrated recurrent translocations and/or coding mutations in a large number of lysine methyltransferases, including MLL family members, MMSET, and EZH2.

MUTATIONS IN HISTONE PROTEINS

Histones H2A, H2B, H3 and H4 are core components of nucleosome, while H1 is a linker histone. In recent years there has been a growing evidence that genetic alterations in a gene coding for H3 itself are frequent driver mutations in pediatric malignant gliomas [59-61]. Mutations in H3 histone coding genes occur most frequently in *H3F3A* and *HIST1H3B* genes and infrequently in the *HIST2H3C* gene [60]. The frequently altered position in the H3 histone is in a H3K27 position, which causes alterations in the H3K27 methylation and acetylation pattern in mutated cells. H3K27 tri-methylation is a crucial histone repression mark important for cell differentiation and maturation. H3K27me3 is carried on by the EZH2 methyl-

transferase, being a part of the PRC2 complex, combined with demethylases JMJD3/KDM6B and UTX/KDM6A. Histone H3K27M alteration leads to inhibition of PRC2 complex, and in consequence, to a global reduction of H3K27 methylation (Fig. 1A). Moreover, nucleosomes bearing H3K27M are also acetylated and recruit BRD4 – a member of BET family of proteins, which bind to acetylated histones. This promotes an open chromatin structure, which favors increased gene transcription (Fig. 1A) [62].

Another mutation found in pediatric gliomas occurs in H3 histone coding genes at a G34 site. The role of G34 variant is still elusive, but in a close proximity to that site is K36 which could be epigenetically modified. Alteration in the G34 position may affect H3K36 methylation pattern (Fig. 1B) [61]. In high-grade gliomas, G34 alteration almost always occurs with *ATRX* or *DAXX* and *TP53* mutations [62,63].

EPIGENETIC LANDSCAPE DEREGULATION

Deregulation of histone modifications in gliomas became an area of intensive investigation. Immunohistochemical analysis of the global H3K4me2, H4K20me3, H3K9Ac, H3K18Ac, H3K9me3 and H4K20me3 glioma samples demonstrated that some histone modifications correlate with the progression-free and overall survival of glioma patients. The lower H3K18Ac and higher H4K20me3 levels correlated with the greater survival of glioblastoma (GBM) patients. H4K20me3 expression was higher in grade II than in normal brain or high grades, whereas H3K9me3 immunoreactivity was higher in normal brain. H3K9me3-positive grade II oligodendrogliomas had improved overall survival compared with H3K9me3-negative cases. The analysis of the phosphorylation level of histone H3 at the tyrosine (T)3,6, serine (S)10, 28 and threonine (Y)41 in a collection of GBM samples demonstrated that the high levels of the phosphorylated histone H3 at the residues T6, S10 and/or T41 are associated with a poor prognosis of GBM patients [46].

The importance of DNA methylation pattern for glioma patients in TCGA datasets, has been highlighted by different methylation pattern *IDH1/IDH2* mutated patients [64, 65]. Mutations in isocitrate dehydrogenase 1 (*IDH1*) gene, coding for the NADP-positive dependent enzyme IDH, occur in ~80% of grade II gliomas and secondary GBMs [66]. Those are recurrent mutations in a range of myeloid malignancies, most notably AML [67]. Mutant IDH1 (also rarely IDH2) acquires an alternative activity, catalyzing the conversion of α-ketoglutarate (α-KG) to 2-hydroxyglutarate (2-HG), which is an "oncometabolite". 2-HG accumulates and competitively inhibits α-KG dependent enzymes, such as histone demethylases and a DNA demethylase TET2. The outcome in affected cells is DNA hypermethylation known as a Glioma CpG Island Methylator Phenotype - G-CIMP [68]. Information about IDH status is the most important diagnostic distinction in the current WHO recommended guideline. IDH-mutated patients have generally better prognosis than patients without mutation. We have demonstrated that specific sites are differentially methylated in glioma patients with long overall survival versus patients with short overall survival [69]. Methylation at this site was a better prognostic factor than IDH status in the cohort of patients from the TCGA repository. Our finding suggests that methylation of DNA could be incorporated in clinical decision making process.

DEREGULATION OF NUCLEOSOME REMODELING

Recent human genetic screens have revealed that genes *BRG1, ARID1A, BAF155,* and *hSNF5* are frequently mutated in tumors, indicating that BAF complexes contribute to the initiation or progression of cancer. In gliomas (brain tumors of the glial lineage), mutations have been found in genes *ATRX, ARID1A*, *SMARCA4*, *SMARCA2*, and *SMARCC2*. The most frequently mutated chromatin remodeler is *ATRX*, frequently mutated in pediatric glioblastomas. Genes *ATRX* (α-thalassaemia/mental retardation syndrome X-linked) and *DAXX* (death-domain associated protein) encode two subunits of a chromatin remodeling complex required for histone variant H3.3 incorporation at pericentromeric heterochromatin and telomeres. ATRX protein is localized at telomeres, pericentromeric heterochromatin, and the inactive X chromosome, where it may regulate the establishment and/or maintenance of transcriptionally silent chromatin. It regulates telomere function, DNA methylation and sister chromatid cohesion by controling chromosome dynamics [70]. It is not exactly known how ATRX deletion contributes to glioma formation. *ATRX* mutations are directly involved in tumorigenesis via alterations at the chromatin level. A recent study indicates that ATRX facilitates the chromatin reconstitution required for DNA repair [71]. ATRX defect is an alternative way of telomere elongation [72]. ATRX inactivation alters chromatin state by shifting or altering deposition of histone variants H3.3 in the genome that in the consequence may lead to aberrant expression of genes involved in differentiation of neural precursor cells into mature glial cells [72].

DRUG DEVELOPMENT

HDAC inhibitors (valproic acid, SAHA) and DNA hypomethylating agents (5-aza-2'-deoxycytidine), represent the only two classes of epigenetic enzymes inhibitors currently approved by the FDA. However, the clinical utility of these drugs has been limited by several factors including chemical instability, a lack of specificity leading to increased genomic instability, DNA damage and cytotoxicity, and significant off-target effects.

Inhibition of individual readers of epigenetic modifications is considered to be more specific and exert less off-target toxicity. Recently bromodomain-containing reader proteins have emerged as attractive targets for cancer drug discovery, especially in hematological malignancies or pediatric H3K27M mutant glioblastoma. JQ1 is the early prototypical bromodomain inhibitors. Treatment of AML cells with JQ1 resulted in potent antiproliferative effects and myeloid differentiation. Multiple bromodomain inhibitors have been developed with enhanced pharmacokinetic properties, including CPI-0610,

OTX015, TEN-010, and I-BET762. These compounds are under investigation in early phase clinical trials for lymphoma, leukemia, and solid tumors [58]. Treatment of pediatric diffuse intrinsic pontine glioma (DIPG) xenografts with JQ1 reduced tumor growth and extended survival. DIPGs bearing K27M mutation in histone H3 depend on residual PRC2 activity and recent data suggest that EZH2, JMJD3/UTX or BET inhibitors may be beneficial in patients with H3K27M-expressing tumors. Drugs targeting epigenetic modifiers, including the inhibitors of histone methyltransferases, demethylases, HDACs and BET proteins, have emerged recently in clinical trials for these tumors [73].

A CpG Island Methylator Phenotype has been associated with several cancers, including leukemia and glioma. Identification of IDH mutations (*mIDH*) associated with a hypermethylated phenotype led to an intensive search for drugs that could interfere with this pathway. Treatments specifically or indirectly targeted to m*IDH* are currently under clinical investigation. Interestingly, these therapies are generally well tolerated and, when used as a single agent induced positive effects in m*IDH* patients in AML. This is evidenced by the recent approval of the first selective mutant IDH2 inhibitor AG-221 (enasidenib) for the treatment of IDH2-mutated AML [74].

CONCLUSIONS

Growing evidence indicates that epigenetic mechanisms are often deregulated in cancer as a cause or a consequence of the malignancy. Driver mutations in genes coding for core histone proteins lead to blockade of repressive K27me3 or in IDH1/2 coding genes affect DNA methylation, which results in deregulated expression of many genes controlling cell proliferation, differentiation and tumorigenesis. Deregulation of epigenetic mechanisms is a common feature of cancer and therapeutic targeting of epigenetic enzymes is one of the emerging strategies for cancer therapy.

REFERENCES

1. Wang GG, Allis CD, Chi P (2007) Chromatin remodeling and cancer, Part I: Covalent histone modifications. Trends Mol Med 13: 363-372
2. Campbell MJ, Turner BM (2013) Altered histone modifications in cancer. Adv Exp Med Biol 754: 81-107
3. Chi P, Allis CD, Wang GG (2010) Covalent histone modifications--miswritten, misinterpreted and mis-erased in human cancers. Nat Rev Cancer 10: 457-469
4. Klose RJ, Bird AP (2006) Genomic DNA methylation: the mark and its mediators. Trends Biochem Sci 31: 89-97
5. Bergman Y, Cedar H (2013) DNA methylation dynamics in health and disease. Nat Struct Mol Biol 20: 274-281
6. Turner BM (2012) The adjustable nucleosome: an epigenetic signaling module. Trends Genet 28: 436-444
7. Tan M, Luo H, Lee S, Jin F, Yang JS, Montellier E, Buchou T, Cheng Z, Rousseaux S, Rajagopal N *et al*. (2011) Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification. Cell 146: 1016-1028
8. Kouzarides T (2007) Chromatin modifications and their function. Cell 128: 693-705
9. Bannister AJ, Kouzarides T (2011) Regulation of chromatin by histone modifications. Cell Res 21: 381-395
10. Waldmann T, Schneider R (2013) Targeting histone modifications--epigenetics in cancer. Curr Opin Cell Biol 25: 184-189
11. Vincenzo DC, Fabio M, P RD, Montellier Emilie , Salim K, Philipp T, Eleni K, Monika H, Leslie H, Angelika F *et al*. (2014) Acetylation of histone H3 at lysine 64 regulates nucleosome dynamics and facilitates transcription. Elife 25: e01632
12. Yun M, Wu J, Workman JL, Li B (2011) Readers of histone modifications. Cell Res 21: 564-578
13. Maleszewska M, Steranka A, Kaminska B (2014) The effects of selected inhibitors of histone modifying enzyme on C6 glioma cells. Pharmacol Rep 66: 107-113
14. Yang XJ, Seto E (2007) HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. Oncogene 26: 5310-5318
15. Johnstone RW, Licht JD (2003) Histone deacetylase inhibitors in cancer therapy: is transcription the primary target? Cancer Cell 4: 13-18
16. Bereshchenko OR, Gu W, Dalla-Favera R (2002) Acetylation inactivates the transcriptional repressor BCL6. Nat Genet 32: 606-613
17. Lucio-Eterovic AK, Cortez MA, Valera ET, Motta FJ, Queiroz RG, Machado HR, Carlotti CG, Neder L, Scrideli CA, Tone LG (2008) Differential expression of 12 histone deacetylase (HDAC) genes in astrocytomas and normal brain tissue: class II and IV are hypoexpressed in glioblastomas. BMC Cancer 8: 243
18. Black JC, Van Rechem C, Whetstine JR (2012) Histone lysine methylation dynamics: establishment, regulation, and biological impact. Mol Cell 48: 491-507
19. Chang B, Chen Y, Zhao Y, Bruick RK (2007) JMJD6 is a histone arginine demethylase. Science 318: 444-447
20. Mantri M, Krojer T, Bagg EA, Webby CJ, Butler DS, Kochan G, Kavanagh KL, Oppermann U, McDonough MA, Schofield CJ (2010) Crystal structure of the 2-oxoglutarate- and Fe(II)-dependent lysyl hydroxylase JMJD6. J Mol Biol 401: 211-222
21. Webby CJ, Wolf A, Gromak N, Dreger M, Kramer H, Kessler B, Nielsen ML, Schmitz C, Butler DS, Yates JR, *et al*. (2009) Jmjd6 catalyses lysyl-hydroxylation of U2AF65, a protein associated with RNA splicing. Science 325: 90-93
22. Zhang R, Wang R, Chang H, Wu F, Liu C, Deng D, Fan W (2012) Downregulation of Ezh2 expression by RNA interference induces cell cycle arrest in the G0/G1 phase and apoptosis in U87 human glioma cells. Oncol Rep 28: 2278-2284
23. Zhang W, Lv S, Liu J, Zang Z, Yin J, An N, Yang H, Song Y (2014) PCI-24781 down-regulates EZH2 expression and then promotes glioma apoptosis by suppressing the PIK3K/Akt/mTOR pathway. Genet Mol Biol 37: 716-724
24. Fan TY, Wang H, Xiang P, Liu YW, Li HZ, Lei BX, Yu M, Qi ST (2014) Inhibition of EZH2 reverses chemotherapeutic drug TMZ chemosensitivity in glioblastoma. Int J Clin Exp Pathol 7: 6662-6670
25. Ott M, Litzenburger UM, Sahm F, Rauschenbach KJ, Tudoran R, Hartmann C, Marquez VE, von Deimling A, Wick W, Platten M (2012) Promotion of glioblastoma cell motility by enhancer of zeste homolog 2 (EZH2) is mediated by AXL receptor kinase. PLoS One 7: e47663
26. Smits M, Nilsson J, Mir SE, van der Stoop PM, Hulleman E, Niers JM, de Witt Hamer PC, Marquez VE, Cloos J, Krichevsky AM *et al*. (2010) miR-101 is down-regulated in glioblastoma resulting in EZH2-induced proliferation, migration, and angiogenesis. Oncotarget 1: 710-720
27. Natsume A, Ito M, Katsushima K, Ohka F, Hatanaka A, Shinjo K, Sato S, Takahashi S, Ishikawa Y, Takeuchi I *et al*. (2013) Chromatin regulator PRC2 Is a key regulator of epigenetic plasticity in glioblastoma. Cancer Res 73: 4559-4570
28. Suvà ML, Riggi N, Janiszewska M, Radovanovic I, Provero P, Stehle JC, Baumer K, Le Bitoux MA, Marino D, Cironi L *et al*. (2009) EZH2 is essential for glioblastoma cancer stem cell maintenance. Cancer Res 69: 9211-9218
29. Lee J, Son MJ, Woolard K, Donin NM, Li A, Cheng CH, Kotliarova S, Kotliarov Y, Walling J, Ahn S *et al*. (2008) Epigenetic-mediated dysfunction of the bone morphogenetic protein pathway inhibits differentiation of glioblastoma-initiating cells. Cancer Cell 13: 69-80

30. Ning X, Shi Z, Liu X, Zhang A, Han L, Jiang K, Kang C, Zhang Q (2015) DNMT1 and EZH2 mediated methylation silences the microRNA-200b/a/429 gene and promotes tumor progression. Cancer Lett 359: 198-205
31. Pyo JS, Kang DW (2018) Prognostic role of EZH2 in gliomas: a meta-analysis. Int J Biol Markers 33: 62-67
32. Spyropoulou A, Gargalionis A, Dalagiorgou G, Adamopoulos C, Papavassiliou KA, Lea RW, Piperi C, Papavassiliou AG (2014) Role of histone lysine methyltransferases SUV39H1 and SETDB1 in gliomagenesis: modulation of cell proliferation, migration, and colony formation. Neuromolecular Med 16: 70-82
33. Ciechomska IA, Przanowski P, Jackl J, Wojtas B, Kaminska B (2016) BIX01294, an inhibitor of histone methyltransferase, induces autophagy-dependent differentiation of glioma stem-like cells. Sci Rep 6: 38723
34. Wang S, Tan X, Yang B, Yin B, Yuan J, Qiang B, Peng X (2012) The role of protein arginine-methyltransferase 1 in gliomagenesis. BMB Rep 45: 470-475
35. Han X, Li R, Zhang W, Yang X, Wheeler CG, Friedman GK, Province P, Ding Q, You Z, Fathallah-Shaykh HM *et al*. (2014) Expression of PRMT5 correlates with malignant grade in gliomas and plays a pivotal role in tumor growth in vitro. J Neurooncol 118: 61-72
36. Yan F, Alinari L, Lustberg ME, Martin LK, Cordero-Nieves HM, Banasavadi-Siddegowda Y, Virk S, Barnholtz-Sloan J, Bell EH, Wojton J *et al*. (2014) Genetic validation of the protein arginine methyltransferase PRMT5 as a candidate therapeutic target in glioblastoma. Cancer Res 74: 1752-1765
37. Sareddy GR, Nair BC, Krishnan SK, Gonugunta VK, Zhang QG, Suzuki T, Miyata N, Brenner AJ, Brann DW, Vadlamudi RK (2013) KDM1 is a novel therapeutic target for the treatment of gliomas. Oncotarget 4: 18-28
38. Dai B, Hu Z, Huang H, Zhu G, Xiao Z, Wan W, Zhang P, Jia W, Zhang L (2014) Overexpressed KDM5B is associated with the progression of glioma and promotes glioma cell growth via downregulating p21. Biochem Biophys Res Commun 454: 221-227
39. Pacaud R, Cheray M, Nadaradjane A, Vallette FM, Cartron PF (2015) Histone H3 phosphorylation in GBM: a new rational to guide the use of kinase inhibitors in anti-GBM therapy. Theranostics 5: 12-22
40. Dawson MA, Kouzarides T, Huntly BJ (2012) Targeting epigenetic readers in cancer. N Engl J Med 367: 647-657
41. Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, Ghosh D, Pienta KJ, Sewalt RG, Otte AP *et al*. (2002) The polycomb group protein EZH2 is involved in progression of prostate cancer. Nature 419: 624-629
42. Kleer CG, Cao Q, Varambally S, Shen R, Ota I, Tomlins SA, Ghosh D, Sewalt RG, Otte AP, Hayes DF *et al*. (2003) EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. Proc Natl Acad Sci USA 100: 11606-11611
43. Ernst T, Chase AJ, Score J, Hidalgo-Curtis CE, Bryant C, Jones AV, Waghorn K, Zoi K, Ross FM, Reiter A *et al*. (2010) Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. Nat Genet 42: 722-726
44. Nikoloski G, Langemeijer SM, Kuiper RP, Knops R, Massop M, Tönnissen ER, van der Heijden A, Scheele TN, Vandenberghe P, de Witte T *et al*. (2010) Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. Nat Genet 42: 665-667
45. van Haaften G, Dalgliesh GL, Davies H, Chen L, Bignell G, Greenman C, Edkins S, Hardy C, O'Meara S, Teague J *et al*. (2009) Somatic mutations of the histone H3K27 demethylase gene UTX in human cancer. Nat Genet 41: 521-523
46. Maleszewska M, Kaminska B (2015) Deregulation of histone-modifying enzymes and chromatin structure modifiers contributes to glioma development. Future Oncol 11: 2587-2601
47. Chen T, Li E (2004) Structure and function of eukaryotic DNA methyltransferases. Curr Top Dev Biol 60: 55-89
48. Bourc'his D, Xu GL, Lin CS, Bollman B, Bestor TH (2001) Dnmt3L and the establishment of maternal genomic imprints. Science 294: 2536-2539
49. Hata K, Okano M, Lei H, Li E (2002) Dnmt3L cooperates with the Dnmt3 family of de novo DNA methyltransferases to establish maternal imprints in mice. Development 129: 1983-1993
50. Goll MG, Kirpekar F, Maggert KA, Yoder JA, Hsieh CL, Zhang X, Golic KG, Jacobsen SE, Bestor TH (2006) Methylation of tRNAAsp by the DNA methyltransferase homolog Dnmt2. Science 311: 395-398
51. Ley TJ, Ding L, Walter MJ, McLellan MD, Lamprecht T, Larson DE, Kandoth C, Payton JE, Baty J, Welch J *et al*. (2010) DNMT3A mutations in acute myeloid leukemia. N Engl J Med 363: 2424-2433
52. Yan XJ, Xu J, Gu ZH, Pan CM, Lu G, Shen Y, Shi JY, Zhu YM, Tang L, Zhang XW *et al*. (2011) Exome sequencing identifies somatic mutations of DNA methyltransferase gene DNMT3A in acute monocytic leukemia. Nat Genet 43: 309-315
53. Simó-Riudalbas L, Melo SA, Esteller M (2011) DNMT3B gene amplification predicts resistance to DNA demethylating drugs. Genes Chromosomes Cancer 50: 527-534
54. Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ, Robertson A, Hoadley K, Triche TJ, Laird PW, Baty JD *et al*. (2013) Genomic and epigenomic landscapes of adult *de novo* acute myeloid leukemia. N Engl J Med 368: 2059-2074
55. Yang L, Rau R, Goodell MA (2015) DNMT3A in haematological malignancies. Nat Rev Cancer 15: 152-165
56. Delhommeau F, Dupont S, Della Valle V, James C, Trannoy S, Massé A, Kosmider O, Le Couedic JP, Robert F, Alberdi A *et al*. (2009) Mutation in TET2 in myeloid cancers. N Engl J Med 360: 2289-2301
57. Weissmann S, Alpermann T, Grossmann V, Kowarsch A, Nadarajah N, Eder C, Dicker F, Fasan A, Haferlach C, Haferlach T *et al*. (2012) Landscape of TET2 mutations in acute myeloid leukemia. Leukemia 26: 934-942
58. Cai SF, Chen CW, Armstrong SA (2015) Drugging chromatin in cancer: recent advances and novel approaches. Mol Cell 60: 561-570
59. Wu G, Diaz AK, Paugh BS, Rankin SL, Ju B, Li Y, Zhu X, Qu C, Chen X, Zhang J *et al*. (2014) The genomic landscape of diffuse intrinsic pontine glioma and pediatric non-brainstem high-grade glioma. Nat Genet 46: 444-450
60. Castel D, Philippe C, Calmon R, Le Dret L, Truffaux N, Boddaert N, Pagès M, Taylor KR, Saulnier P, Lacroix L *et al*. (2015) Histone H3F3A and HIST1H3B K27M mutations define two subgroups of diffuse intrinsic pontine gliomas with different prognosis and phenotypes. Acta Neuropathol 130: 815-827
61. Korshunov A, Capper D, Reuss D, Schrimpf D, Ryzhova M, Hovestadt V, Sturm D, Meyer J, Jones C, Zheludkova O *et al*. (2016) Histologically distinct neuroepithelial tumors with histone 3 G34 mutation are molecularly similar and comprise a single nosologic entity. Acta Neuropathol 131: 137-146
62. Lulla RR, Saratsis AM, Hashizume R (2016) Mutations in chromatin machinery and pediatric high-grade glioma. Sci Adv 2: e1501354
63. Fontebasso AM, Schwartzentruber J, Khuong-Quang DA, Liu XY, Sturm D, Korshunov A, Jones DT, Witt H, Kool M, Albrecht S *et al*. (2013) Mutations in SETD2 and genes affecting histone H3K36 methylation target hemispheric high-grade gliomas. Acta Neuropathol 125: 659-669
64. Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, Berman BP, Pan F, Pelloski CE, Sulman EP, Bhat KP *et al*. (2010) Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell 17: 510-522
65. Brat DJ, Verhaak RG, Aldape KD, Yung WK, Salama SR, Cooper LA, Rheinbay E, Miller CR, Vitucci M, Morozova O *et al*. (2015) Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. N Engl J Med 372: 2481-2498
66. Maleszewska M, Kaminska B (2013) Is glioblastoma an epigenetic malignancy? Cancers (Basel) 5: 1120-1139
67. Rakheja D, Konoplev S, Medeiros LJ, Chen W (2012) IDH mutations in acute myeloid leukemia. Hum Pathol 43: 1541-1551
68. Turcan S, Rohle D, Goenka A, Walsh LA, Fang F, Yilmaz E, Campos C, Fabius AW, Lu C, Ward PS *et al*. (2012) IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. Nature 483: 479-483

69. Dabrowski MJ, Draminski M, Diamanti K, Stepniak K, Mozolewska MA, Teisseyre P, Koronacki J, Komorowski J, Kaminska B, Wojtas B (2018) Unveiling new interdependencies between significant DNA methylation sites, gene expression profiles and glioma patients survival. Sci Rep 8: 4390

70. Kadoch C, Crabtree GR (2015) Mammalian SWI/SNF chromatin remodeling complexes and cancer: Mechanistic insights gained from human genomics. Sci Adv 1: e1500447

71. Ceccarelli M, Barthel FP, Malta TM, Sabedot TS, Salama SR, Murray BA, Morozova O, Newton Y, Radenbaugh A, Pagnotta SM *et al*. (2016) Molecular Profiling Reveals Biologically Discrete Subsets and Pathways of Progression in Diffuse Glioma. Cell 164: 550-563

72. Danussi C, Bose P, Parthasarathy PT, Silberman PC, Van Arnam JS, Vitucci M, Tang OY, Heguy A, Wang Y, Chan TA *et al*. (2018) Atrx inactivation drives disease-defining phenotypes in glioma cells of origin through global epigenomic remodeling. Nat Commun 9: 1057

73. Hashizume R (2017) Epigenetic Targeted Therapy for Diffuse Intrinsic Pontine Glioma. Neurol Med Chir (Tokyo) 57: 331-342

74. Sharma H (2018) Development of Novel Therapeutics Targeting Isocitrate Dehydrogenase Mutations in Cancer. Curr Top Med Chem 18: 505-524

Deregulacja mechanizmów epigenetycznych w nowotworach

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Słowa kluczowe: nowotwory, epigenetyka, histon, modyfikacje histonów, metylacja, chromatyna

STRESZCZENIE

Ekspresja genów w komórkach jest ściśle kontrolowana i podlega regulacji m.in. za pomocą mechanizmów epigenetycznych takich jak metylacja DNA, modyfikacje histonów, pozycjonowanie nukleosomów czy niekodujące RNA. Deregulacja mechanizmów epigenetycznych odgrywa kluczową rolę w procesie nowotworzenia, przy czym naukowcy spierają się czy zjawisko to jest przyczyną czy też konsekwencją rozwoju nowotworu. W wyniku deregulacji mechanizmów epigenetycznych w komórce następują głębokie zmiany w ekspresji genów zarówno na poziomie RNA jak i białka. W wyniku zmian epigenetycznych dochodzi najczęściej do zwiększonej proliferacji komórek, wzrostu nowotworu i przerzutowania. Większość zmian epigenetycznych w komórce takich jak nieprawidłowe wzory metylacji DNA czy modyfikacji histonów jest wynikiem mutacji w genach odpowiedzialnych za ich wprowadzanie lub usuwanie. Mutacje w genach regulujących pozycjonowanie nukleosomów prowadzą do zmian w organizacji genomu w jądrze, a w konsekwencji do mutacji na poziomie chromosomu, takich jak translokacje czy zmiany liczby kopii genu w komórce. Ponadto, zaobserwowano również mutacje w genach metabolizmu komórkowego, które mogą wpływać na aktywność genów odpowiedzialnych za demetylację DNA lub modyfikacje histonów. Rozwój technik sekwencjonowania cało genomowego umożliwił poznanie globalnych zmian w profilu epigenetycznym komórek nowotworowych.