Derleme Makalesi / *Review Article*

İZTÜ Tıp ve Sağlık Bilimleri Dergisi (*IZTU Journal of Medical and Health Sciences*) 1 (1): 35-45, 2022

MEDULLOBLASTOMLARDAKİ EPİGENETİK DEĞİŞİKLİKLERİN MOLEKÜLER ALT GRUPLARI İLE İLİŞKİSİ

EPIGENETIC CHANGES IN MEDULLOBLASTOMA: CORRELATION WITH MOLECULAR SUBCLASSIFICATION

Naz KANIT[1] Erdener OZER[1,2]

[1] Dokuz Eylul University Institute of Health Sciences, Department of Molecular Medicine, Balcova, Izmir/Turkey

[2] Dokuz Eylul University School of Medicine, Department of Pathology, Balcova, Izmir/Turkey

Anahtar Sözcükler: DNA metilasyonu, histon modifikasyonları, kromatin yeniden düzenlenmesi, lncRNA, medulloblastom

Keywords: Medulloblastoma, DNA methylation, histone modifications, chromatin remodeling, lncRNA

ÖZ

Medulloblastom (MB) çocukluk çağının malign beyin tümörü olmakla beraber klinik heterojenitesi oldukça yüksektir. Histolojik alt sınıflandırma yanısıra; moleküler olarak WNT-aktive, SHH-aktive ve WNT/SHH-aktive-olmayan üzere üç temel alt grubu tanımlanmıştır. Son grup, Grup 3 ve Grup 4 medulloblastomları içermektedir. Tüm gruplar, farklı histolojik tiplerin yanı sıra, farklı genetik ve epigenetik özellikler gösterebilmektedir. Geçtiğimiz on yılda hastalığın genetik yapısı detaylı bir şekilde incelenmiştir, ancak epigenetik temelleri son zamanlarda araştırma odağı olmuştur. Epigenetik araştırmalar KDM6A ve EZH2 gibi genler üzerinden histon modifikasyon mekanizmaları, PRC2 kompleksi ve başta SWI/SNF kompleksi olmak üzere ATP-bağımlı kromatin yeniden-düzenleyici kompleksleri üzerine yoğunlaşmıştır. EZH2 geninin baskılayıcıları günümüzde klinik denemelerde MB hastaları üzerinde test edilmekte olup bu gen aday hedef genlerden biridir. Son olarak, kodlamayan RNA'lardan lncRNA'ların alt gruplara özgü belirteçler arasında en umut verici belirteçler olacağı tahmin edilmektedir. Medulloblastomlardaki genetik ve epigenetik farklılıkları anlamak, alt gruplara özgü değişiklikleri tanımlamak ve bu değişiklikleri hedefleyen terapötiklerin ortaya çıkarılması, bu kanserin tedavisinde oldukça önemli olacaktır. Bu derlemede amacımız, medulloblastomlardaki epigenetik değişiklikleri güncel literatür ile irdelemek ve konuyla ilişkili yürüttüğümüz çalışmadaki ön verilerimizi ortaya koymaktır.

SUMMARY

Medulloblastoma is a malignant childhood brain tumor and shows high clinical heterogeneity among patients. Three major molecular categories of MB have been established; WNT activated group, SHH activated group, and non-WNT/non-SHH-activated group. The latter includes Group 3 and Group 4. All groups show different histological features as well as different genetic and epigenetic backgrounds. Genetic basis of the disease has been widely studied in the last decade, however epigenetic basis of the disease has become a trend research area. The epigenetic researches focus on histone modification mechanisms involving some genes such as KDM6A and EZH2, and also PRC2 complex, in addition to variations in ATP-dependent chromatin remodeling complexes, mainly on SWI/SNF complexes. EZH2 is a candidate target gene as its repressors are currently on trial for MB patients. Finally lncRNA, a noncoding RNA is likely to be the most promising subgroup specific

marker. Understanding both genetic and epigenetic differences in medulloblastomas, determining subtype-specific alterations and discovering therapeutics that specifically targets those alterations might be valuable for management of this cancer. In this review, we aimed to address the epigenetic mechanisms in medulloblastomas in the light of the current literature and emphasize the relevant unpublished data in our preliminary study.

INTRODUCTION

Medulloblastoma (MB) is an embryonal central nervous system (CNS) tumor that comprises 2% of all primary brain tumors and 18-20% of all childhood brain tumors (1). MB is the most common pediatric CNS tumor, and it is rarely seen in adults. It is usually located in the cerebellum and grows rapidly.

Standard medical management includes surgical resection followed by chemotherapy or radiation therapy. Even though all MB patients are treated in a similar manner, it may show very heterogeneous clinical course. This condition may be explained by the observation that each case has different molecular and histological features (2,3).

Molecular Subgroups

Currently, there are at least four different genetically defined subgroups of MB according to the data achieved by transcriptome analysis, which is the gold standard method (Figure 1). These subgroups are WNT-activated MB, SHH-activated MB, and Non-WNT/Non-SHH MB, namely Group 3 and Group 4 (4,5). All show characteristically different clinical and prognostic features.

1. WNT-activated medulloblastomas

These tumors comprise 10% of all MB cases and have the most favorable prognosis compared to the other subgroups. Survival rate is over 90%. WNT-activated tumors are defined with the abnormal activation of canonical (beta catenin dependent) WNT pathway. The main indicator of this subtype of tumors is the nuclear beta catenin accumulation, which can be detected by immunohistochemistry. Somatic mutations of *CTNNB1* are the most common indicators of this subgroup, as well as monosomy 6. (4–6)

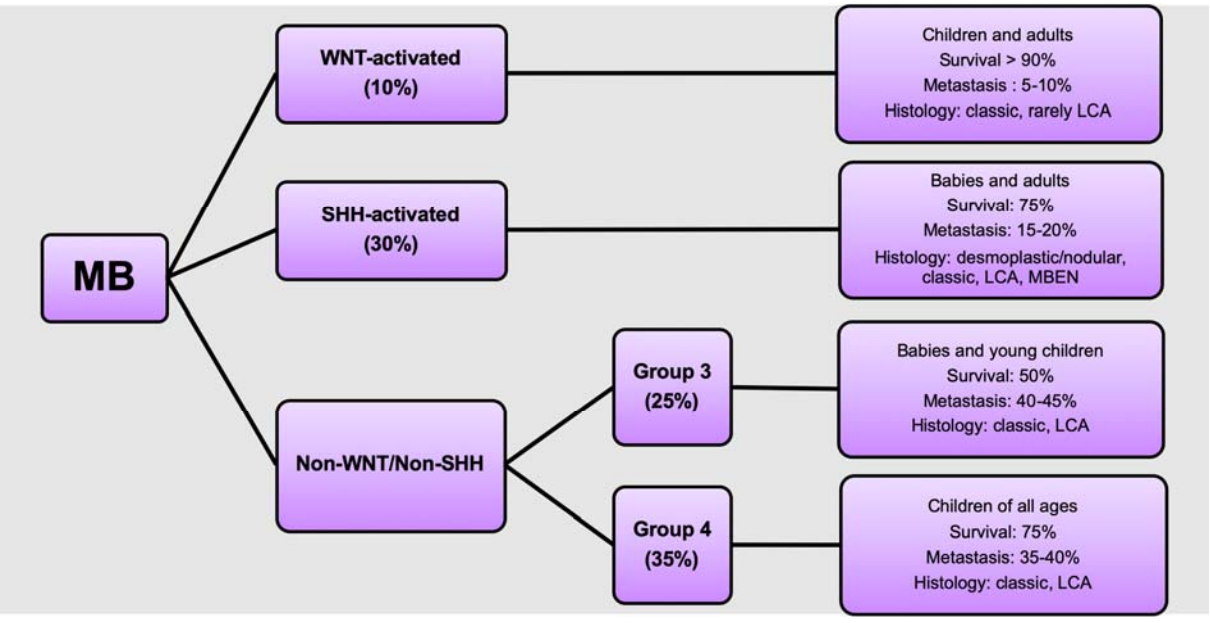

Figure 1. Molecular subclassification of medulloblastoma (LCA: large cell anaplastic)

2. SHH-activated medulloblastomas

This subtype of MB tumors is defined by the abnormal activation of Sonic Hedgehog (SHH) pathway. SHH-activated tumors comprise 30% of all MB cases, and show worse prognosis compared to WNT-activated tumors. Any alterations leading to the activation of SHH pathway, such as somatic mutations of PTCH, SMO, SUFU and amplifications of GLI1 and are the genetic indicators of SHH-activated MB. These group tumors have two distinct subgroups depending on the presence of TP53 mutation. Approximately 20% of all SHH-activated MB shows TP53 mutations, which indicates a worse prognosis. (4,5,7–9)

3. Non-WNT/Non-SHH medulloblastomas

Almost 60% of all MB cases are included in this subgroup, which can also be further subcategorized as Group 3 and Group 4 tumors. There is limited information on the molecular basis of these tumors, and these two subgroups can only be defined with a transcriptome analysis. Twenty-five percent of all MB cases belong to Group 3 subtype, which has the worst prognosis with a 50% survival rate whereas Group 4 MB comprises 35% of all MB cases and has an intermediate prognosis, similar to SHH-activated MB. Because molecular basis of these subgroups is yet to be discovered, there are no well-established molecular indicators. (4,5,7)

Epigenetics

Epigenetics is widely defined as heritable alterations in gene expression activity without any alterations in the DNA sequence (10). The impact of epigenetics on human disease has been known for a long time, and its significance on MB was first shown in 2001 by Frühwald et al who showed the abnormal hypermethylation of some CpG islands which may impact the prognosis of the disease (11). These findings accelerated the studies related to epigenetics in MB and recently a number of researchers have focused exclusively on the epigenetic aspect of the disease. In 2013, Hovestadt et al (12) demonstrated that methylation profiling might be used successfully in subgrouping MB, with over 95% compliance to the gold standard technique of transcriptome analysis.

This observation led the further studies on molecular subgroups of MB, since transcriptome analysis is best performed with fresh frozen tumor tissues, whereas formalin fixed paraffin embedded tissues can be used for methylation profiling studies (12). Current clinical approaches in oncology require analyzing specific indicators with more economic and feasible techniques. Therefore, analyzing the epigenetic changes in MB is crucial for the clinical management of the disease. Epigenetic regulation can occur by four different machineries: methylation of DNA on CpG islands, histone modifications, ATP dependent remodeling of chromatin and via non-coding RNAs.

1. DNA Methylation

This epigenetic mechanism is the most researched area of epigenetics. DNA methylation can be basically described as covalent attachment of a methyl group to a cytosine molecule. This alteration generally prevents transcription factors to bind to the methylated sequences, leading to the silencing of a gene. Methylation and demethylation of DNA is a crucial mechanism of development, and controlled by various DNA methyltransferases (DNMT) and DNA demethylases (10). Abnormal methylation or demethylation of DNA leads to abnormal expression of genes and gene products (Figure 2) (13).

Since the discovery of abnormal DNA methylation patterns in MB (11), researchers have been focused on epigenetic changes, and mostly in DNA methylation patterns. Several genes are shown to be directly related to the prognosis of MB are silenced via promoter hypermethylations. Tumor suppressor genes including *CDKN2A* (11), *RASSF1* (14), *HIC1* (15), *CASP8* (16), *ZIC2* (17), *KLF4* (18), *PTCH1* (19) and *SFRP* family genes (*SFRP1,2,3)* (20) were found to be silenced by promoter hypermethylation in MB.

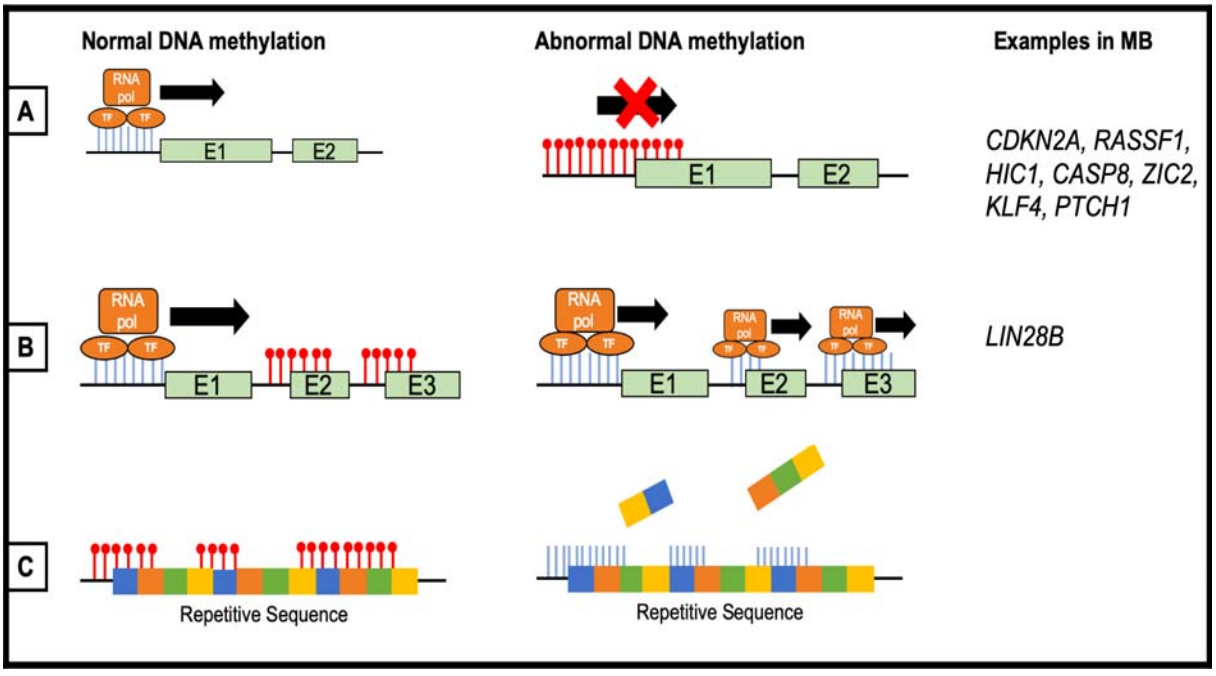

Figure 2. Abnormal methylation and demethylation of DNA may lead to **A)** gene silencing by promoter of promoter-adjacent sequence methylation, which prevents the transcription factors (TF) to bind to target sequences; **B)** abnormal gene expression in account of hypomethylation of DNA, resulting in undesirable RNA residues; **C)** demethylation of repetitive or noncoding sequences, which may consequently cause improper transposition, recombination and genome instability.

Our research on DNA methylation levels of MB showed that high levels of *RASSF1A* hypermethylation was linked to higher occurrence of metastasis whereas *PTCH1* and *ZIC2* methylations were enriched in SHH-active p53-wildtype MBs. In addition, *KLF4* hypermethylation was observed significantly in SHH-activated MB showing no relation with the clinical outcome. We also demonstrated that increased *SPINT2* methylation was present in non-WNT/non-SHH activated MB and might be a potential biomarker for worse prognosis (unpublished data).

Besides these genes, expression of *VAV1*, a general oncogene that has a critical role in tumor maintenance in MB, was shown to be upregulated via hypomethylation (21). Interestingly, in another study, the expression of *LIN28B* gene was upregulated by the hypomethylation of the upstream sequence of the promoter region, improving the aggressiveness of the tumor especially in Group 3 and Group 4 MB (22).

2. Histone modifications

Histones are small, basic proteins that serve in the packaging of DNA by forming histone octomers consisting of two dimers of H2A and H2B histones and two heterodimers of H3-H4 histones, and each histone octomer is linked by H1 histone linker protein. Histones undergo post-translational modifications (PTM), mostly on the positively charged amino acids (such as lysine and arginine) of their free N-terminal tails, allowing changes in the chromatin structure to form euchromatin (can be actively transcribed – open form; generally characterized by high acetylation and H3K4 methylation, H3K36 methylation, H3K79 trimethylation) or heterochromatin (transcriptionally inactive – closed form; generally characterized by low acetylation and H3K9, H3K27, H4K20 methylation), depending on the location and type of the PTM. Histone modifications are crucial for normal cellular activity in different processes such as DNA replication, alternative splicing and DNA repair. (10,13)

Histones can be subjected to many different PTMs, such as acetylation, methylation, phosphorylation, ubiquitination and citrullination (Figure 3A) (10). Histone PTMs are reversible and the effect of PTM depends on the type of the

modification and where the specific modification takes place. Acetylation of lysine residues generally leads to euchromatin structure since the acetyl group neutralizes the positive charge of lysine and relaxes the chromatin structure, enabling transcription factors to interact with the DNA. In addition, the degree of methylation also affects the outcome since lysine amino acids can be mono-, di- or trimethylated. (23)

Histone PTMs are carried out by several specified proteins. Histone acetylation and deacetylation are performed by histone acetyltransferases (HATs) and histone deacetylases (HDACs) (Figure 3B) whereas histone methylation and demethylations are carried out by histone methyltransferases (HMTs) and histone demethylases (HDMs), respectively (Figure 3C) (23). “Writer” proteins are in charge of addition of new histone marks; “eraser” proteins remove the marks and “reader” proteins recognize the specific histone marks and conduct gene transcription when needed (Figure 3D) (24).

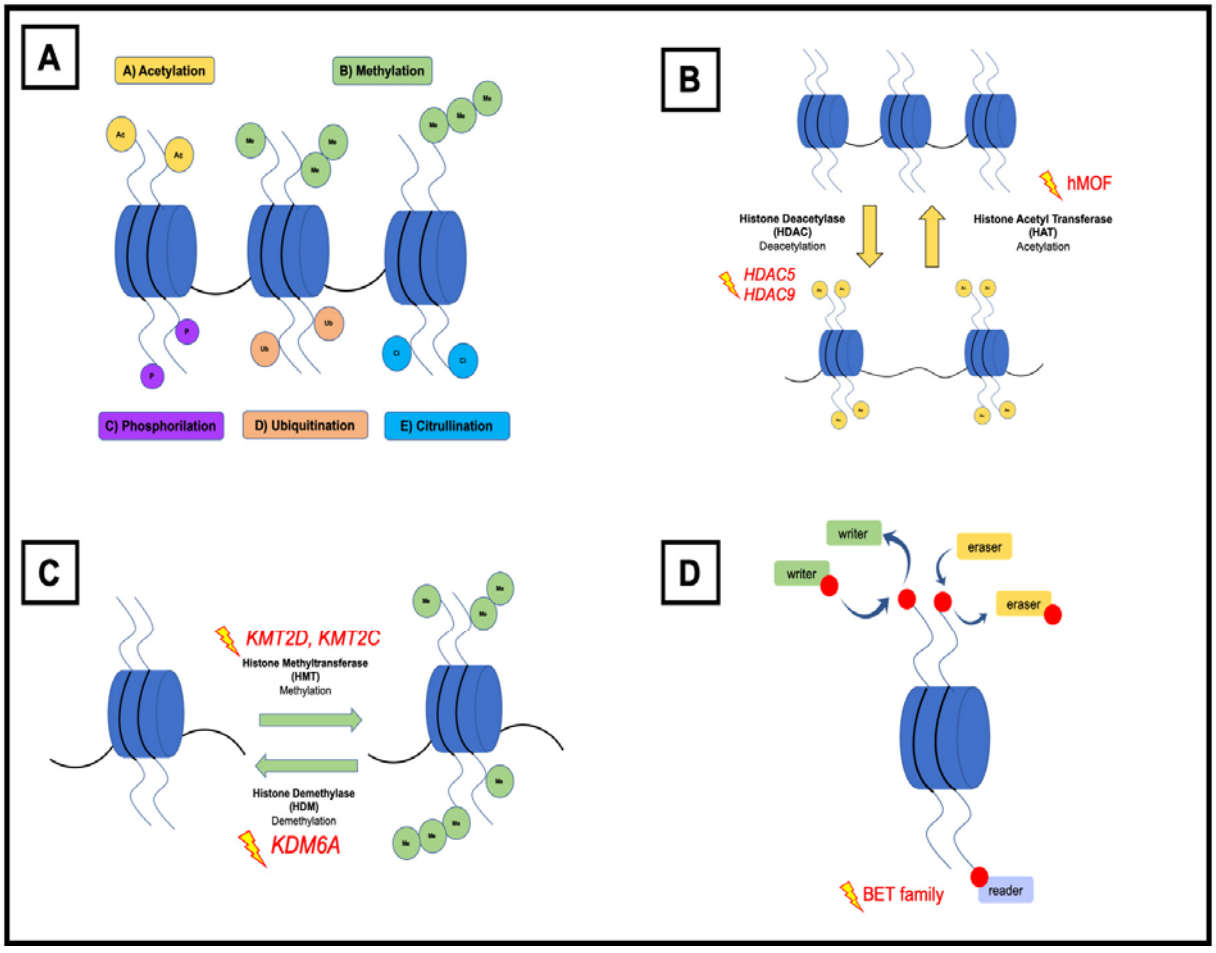

Figure 3: Histone modifications take place on the N-terminal of histone proteins. **A)** Histones are mostly acetylated, mono-, di- or tri- methylated, phosphorylated, ubiquitinated, citrullinated **B)** Histone acetyltransferases (HATs) transfer acetyl groups to histone tails, and these marks are removed by histone deacetylases (HDACs). **C)** Histone mono-, di- or trimethylation is carried out by various histone methyltransferases (HMTs) and these marks are removed or modified by histone demethylases (HDMs). **D)** Histones are modified by “writer” and “eraser” proteins and the generated marks are recognized by “reader” proteins.

Studies show that histone modifications have a great impact on development of MB, and there is a distribution of alterations on several writer, eraser and reader proteins. Sixteen percent of MB (mostly in SHH-activated and Group 4 patients) was found to carry mutations that inactivate the expression of *KMT2D* and *KMT2C* which encode lysine methyltransferases. Activities of both enzymes are related to euchromatin structure and were shown to act as tumor suppressors (25). Alterations that lead to the downregulation of hMOF, the H4K16 HAT were found to affect prognosis of MB patients (26). Similarly, it was shown that upregulation of *HDAC5* and *HDAC9* contribute to the abnormal cell cycle progression across MB subtypes (27). Furthermore, histone demethylation was found to be another important marker in MB inactivating mutations of lysine demethylase *KDM6A*, which occurs commonly in Group 4 MB (28). Additionally, alterations in BET (Bromodomain (BRD) and extraterminal motif containing) family proteins were found in MB. BET family proteins control the transcription of several genes including *MYC*, one of the Group 3 MB driver genes by recognizing and binding to acetylated histone marks and initiation transcription. Alteration of expression of BET family genes could therefore lead to overexpression of *MYC* (29).

Another histone modification mechanism intensely studied on MB is the alterations on polycomb repressor complex (PRC2). It consists of five subunits; EZH2, EED, SUZI2, JARID2 and RBAp46.48 and is in charge of generating H3K27me3 mark which leads to inactivation of transcription via heterochromatin condensation (30). EZH2 is the functional component of the PRC2 complex and some Group 3 and Group 4 MB show increased levels of EZH2, and as a result, H3K27me3 marks. It was also shown that mutations leading to the complete loss of *KDM6A* are observed alongside *EZH2* overexpression, which could be a marker for MB prognosis (31).

3. ATP-dependent chromatin remodeling

ATP-dependent chromatin remodeling is a mechanism that controls nucleosome positioning and the way the DNA molecule is packaged into nucleosomes in numerous ways, thereby controlling expression of genes by either allowing or blocking transcription factors and activators to bind to DNA (13,32). In eukaryotes, this mechanism is performed by four different ATP-dependent chromatin remodeling complexes; ISWI, CHD, SWI/SNF and INO80. Each complex has different ways to perform nucleosome positioning (Figure 4). ISWI (imitation switch) complex control chromatin accessibility by repositioning nucleosomes which either disables access to the DNA, leading to downregulation and silencing of genes, or in contrast, promoting transcription by enabling access to DNA. CHD (Chromodomain helicase DNA-binding) complex contributes to nucleosome positioning in three different ways; by spacing nucleosomes in a pre-determined manner, by exposing promoters via repositioning histones, or by incorporating histone variants to the target site. SWI/SNF (switch/sucrose non-fermentable) complex ejects or slides nucleosomes, thereby promoting chromatin access, leading to activation or repression of target genes. INO80 complex carries out ATP-dependent chromatin remodeling by modifying nucleosomes to grant access to promoters, by organizing nucleosomes, and by replacing histones with specific histone variants. (32)

Defects in ATP-chromatin remodeling mechanisms are widely observed in MB. Especially mutations of SWI/SNF complex proteins are the most studied aspects of this epigenetic mechanism. Studies show that SMARCA4, a member of the SWI/SNF family proteins, is widely mutated in WNT and Group 3 MB (24). It was also found that SMARCA4 has a very important role in development of SHH subtype of MB, coordinating genetic and epigenetic pathways crucial for the development of the tumor (33). Furthermore, mutations in *CHD7,* which codes for another chromatin remodeling protein, were observed in Group 3 and Group 4 MB; and mutations in *DDX3X,* gene which encodes a Dead-Box RNA helicase, was showed to contribute to aberrant WNT signaling and is mostly seen in WNT-activated MB (28).

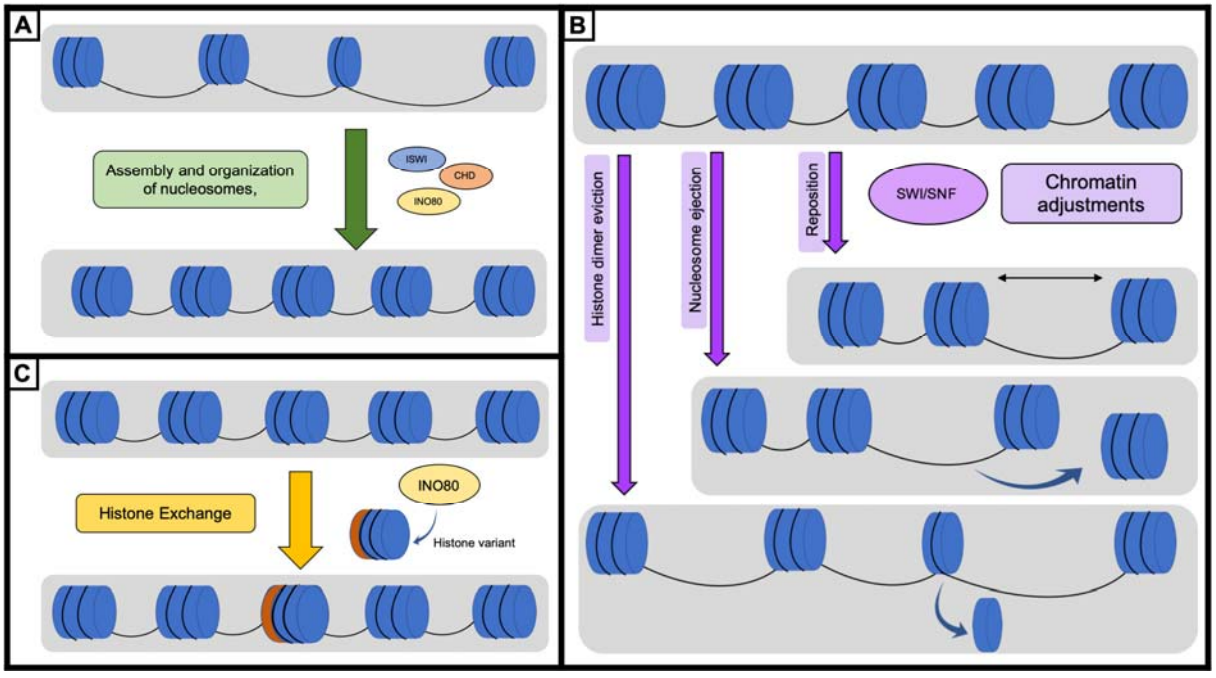

Figure 4. ATP-dependent chromatin remodeling mechanisms. **A)** Maturation of nucleosomes is carried out by ISWI, CHD and INO80 complexes. **B)** SWI/SNF family proteins can reposition nucleosomes and eject histones fully or partially to enable changes in gene expression. **C)** Histone variants can be incorporated to the DNA by INO80 family complexes.

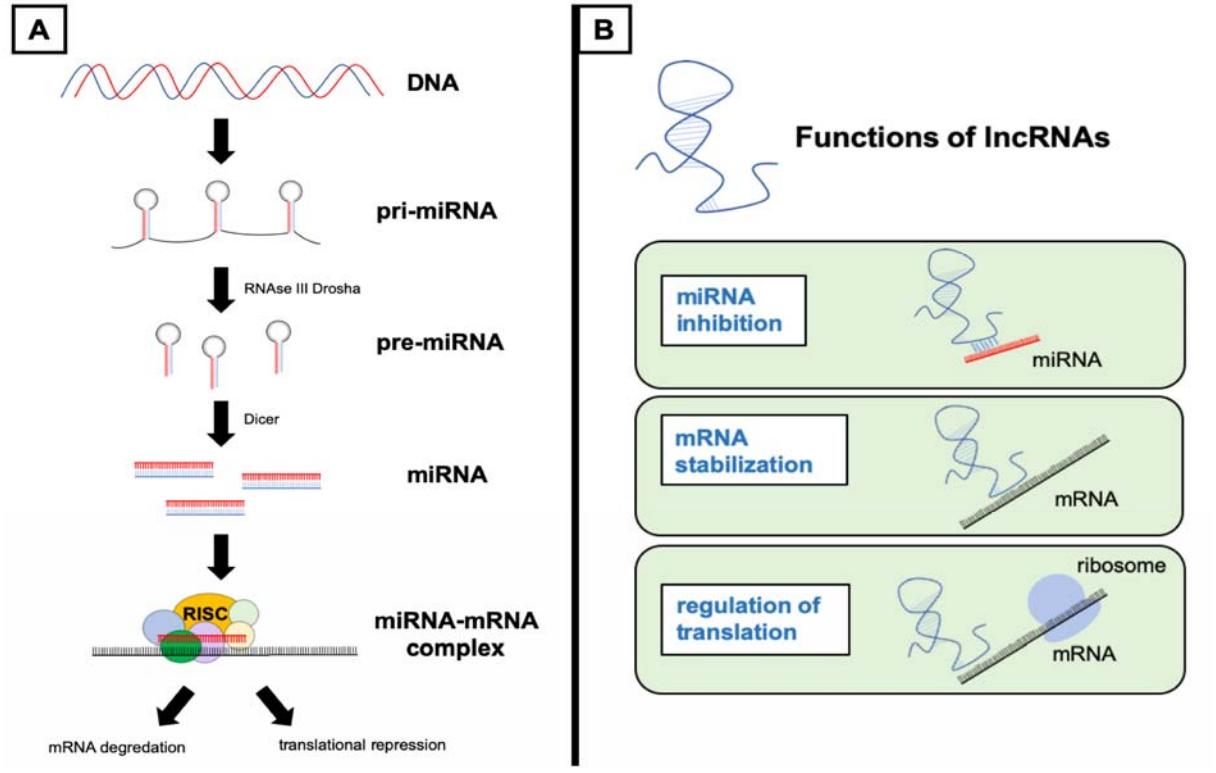

Figure 5. Epigenetic regulation by noncoding RNAs **A)** miRNA is synthesized from DNA as pri-miRNA, maturated to miRNA after consecutive cleaving by Drosha and Dicer enzymes. After binding to RNA-induced silencing complex (RISC), it binds to target mRNA, which is either degraded or transcription is repressed. **B)** lncRNAs are found to participate in various processes such as miRNA inhibition, mRNA stabilization and translational regulation.

Epigenetic mechanisms tend to affect one another, and several alterations go hand in hand in certain situations. Mutations in *ZMYM3,* which codes for a histone binding protein, were observed only in Group 4 MB (34), however they were also commonly seen along with *KDM6A* mutations and decreased *EZH2* expression indicating the cooperation between different epigenetic modifications and its potential importance in MB (28).

4. Non-coding RNAs (miRNAs and lncRNAs)

Non-coding RNAs (ncRNAs) constitute an important part of the eukaryotic DNA and after transcribed, they can have structural and regulatory roles in a viable cell. Apart from messenger RNAs (mRNAs) that code for proteins, all different kinds of RNAs are defined as ncRNAs. Increasing number of studies show that ncRNAs can affect transcription and translation by interacting with epigenetic modulators (23). Studies on MB show that especially miRNAs and lncRNAs have crucial roles in development of MB (25).

The miRNAs are a form of short ncRNAs and consist of 19-25 nucleotides. miRNAs can regulate gene expression directly or indirectly by binding to a target mRNA and repress transcription by cleaving the mRNA or repressing translation process, inhibiting enzymes of epigenetic regulation (such as DNMTs), and interfering with substrates necessary for certain enzymatic reactions (23). miRNAs are transcribed from DNA as long chains, then processed into mature miRNAs in cytoplasm via RNAse III Drosha and Dicer enzymes and form a complex with RNA-induced silencing complex (RISC), and bind to target mRNAs (Figure 5A) (25,35). Since miRNA activity is crucial for a normal cell to function properly, abruption of normal miRNA functions can lead to various diseases.

miRNAs are the most studied ncRNAs in MB, and various alterations were shown to affect the MB progression and prognosis. Tumor suppressor *miR-193* was found to be upregulated in WNT-MB (36). Oncogenic *miR-17~92* cluster, which promotes SHH pathway via increased *MYCN* expression, was found to be upregulated in SHH-MB (37); and *miR-30b/d* with unknown function was shown to be upregulated in Group 3 MB (38). In addition, many other miRNAs were discovered to have altered expression levels throughout MB subtypes. Upregulation of *miR-21* (promotes metastasis via *PDCD4*) (39), *miR-10b* (inhibits apoptosis via *BCL2*) (40), *miR106b* (promotes proliferation via *PTEN*) (41) and downregulation of *miR-124* (42), *miR-125b*, *miR-324* and *miR326* which control cell proliferation (43) were also reported in the relevant literature.

The lncRNAs are ncRNAs longer than 200 nucleotides and are transcribed from the antisense strand of genomic loci by RNA polymerase II (25,44). Some lncRNAs are reported to function as regulators of gene expression in normal cells (Figure 5B), though specific functions of most lncRNA are yet to be known (45).

Studies on lncRNAs on MB shows several subtype-specific lncRNA expression patterns, as well as those indifferent of MB subtypes. One of the first studies on lncRNAs led to the discovery of Linc-NeD125 (also referred to as MIR100HG), which binds three miRNAs from *miR-17~92* cluster, preventing them to repress their target mRNAs. Overexpression of Linc-NeD125 leads to the expression of several major driver genes of Group 4 MB including *KDM6A*, *SNCAIP*, *CDK6* (46). Another lncRNA, NKX2-2-AS1, which has a role to bind miRNAs to suppress several tumor-suppressor genes was found to be downregulated in SHH-MB (47). A recent study on lncRNA profiling of MB subtypes reveals that the lncRNAs expression levels are varied in each subtype of MB. This study showed that upregulation of EMX2OS, LINC01315, LINC00348 and LINC01419 lncRNAs are only observed in WNT, SHH, Group 3 and Group 4 MB, respectively, which can further be investigated for an indicator for subgrouping (48).

CONCLUSION

For the last decade, the studies have shown a significant importance of epigenetic regulators of MB molecular subtypes in correlation with prognosis. Even though many genes have been investigated for the presence of DNA methylations, none of those have been identified

yet as a subgroup specific marker. *KDM6A* down regulation along with *EZH2* overexpression, both related to histone modification is likely to be a specific genetic change for Group 4 MB, however more research is needed to determine its significance. PRC2 complex, another histone modification mechanism is another target for epigenetics research in MB. Additionally, variations in ATP-dependent chromatin remodeling complexes, mainly on SWI/SNF complexes are shown in a number of studies. Finally, various expression levels of many noncoding RNAs were observed in MB such as lncRNAs, the most promising subgroup specific marker.

Determination of any specific biomarker can potentially allow the discovery of targeted therapeutics in MB patients, and help uncover many new epigenetic regulators as drug targets in a wide variety of cancers. *EZH2* is likely to be the candidate target as its repressors are currently on trial for MB patients. Other frequently mutated genes such as *KDM6A* and *DDX3X* are also likely to be targets for future drug researches, allowing wider treatment possibilities for MB patients, in addition to uncovering possible treatment alternatives for other cancers with similar mutations.

REFERENCES

1. Crawford JR, MacDonald TJ, Packer RJ. Medulloblastoma in childhood: new biological advances. Lancet Neurol. 2007 Dec;6(12):1073–85.
2. Louis DN, Perry A, Reifenberger G, Von Deimling A, Figarella-Branger D, Webster, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. 2016;131:803–20.
3. Northcott PA, Korshunov A, Pfister SM, Taylor MD. The clinical implications of medulloblastoma subgroups. Nat Publ Gr. 2012;8:340–51.
4. Taylor MD, Northcott PA, Korshunov A, Remke M, Cho YJ, Clifford SC, et al. Molecular subgroups of medulloblastoma: The current consensus. Acta Neuropathol. 2012;123(4):465–72.
5. Liang L, Aiken C, Felton K, Hogg A, van Landeghem F, Klonisch T, et al. Primary Pediatric Brain Tumors of the Posterior Fossa Part II: A Comprehensive Overview of Medulloblastoma. In: Development of the Cerebellum from Molecular Aspects to Diseases. Springer International Publishing; 2017. p. 327–51.
6. Zurawel RH, Chiappa SA, Allen C, Raffel C. Sporadic medulloblastomas contain oncogenic β-catenin mutations. Cancer Res. 1998;58(5):896–9.
7. Northcott PA, Korshunov A, Witt H, Hielscher T, Eberhart CG, Mack S, et al. Medulloblastoma Comprises Four Distinct Molecular Variants. J Clin Oncol. 2010;29:1408–14.
8. Zhukova N, Ramaswamy V, Remke M, Pfaff E, Shih DJH, Martin DC, et al. Subgroup-Specific Prognostic Implications of TP53 Mutation in Medulloblastoma. J Clin Oncol. 2013;31(23):2927–35.
9. Taylor MD, Mainprize TG, Rutka JT. Molecular insight into medulloblastoma and central nervous system primitive neuroectodermal tumor biology from hereditary syndromes: A review. Neurosurgery. 2000;47(4):888–901.
10. Sadakierska-Chudy A, Kostrzewa RM, Filip M. A Comprehensive View of the Epigenetic Landscape Part I: DNA Methylation, Passive and Active DNA Demethylation Pathways and Histone Variants. Neurotox Res. 2015; 27(1): 84–97
11. Frühwald MC, O'Dorisio MS, Dai Z, Tanner SM, Balster DA, Gao X, et al. Aberrant promoter methylation of previously unidentified target genes is a common abnormality in medulloblastomas–Implications for tumor biology and potential clinical utility. Oncogene. 2001;20(36):5033–42.
12. Hovestadt V, Remke M, Kool M, Pietsch T, Northcott PA, Fischer R, et al. Robust molecular subgrouping and copy-number profiling of medulloblastoma from small amounts of archival tumour material using high-density DNA methylation arrays. Acta Neuropathol. 2013;3:913–6.
13. Portela A, Esteller M. Epigenetic modifications and human disease. Nat Biotechnol. 2010;28(10):1057–68.
14. Lusher ME, Lindsey JC, Latif F, Pearson ADJ, Ellison DW, Clifford SC. Biallelic epigenetic inactivation of the RASSF1A tumor suppressor gene in medulloblastoma development. Cancer Res. 2002;62(20):5906–11.
15. Rood BR, Zhang H, Weitman DM, Cogen PH. Hypermethylation of HIC-1 and 17p allelic loss in medulloblastoma. Cancer Res. 2002;62(13):3794–7.
16. Zuzak TJ, Steinhoff DF, Sutton LN, Phillips PC, Eggert A, Grotzer MA. Loss of caspase-8 mRNA expression is common in childhood primitive neuroectodermal brain tumour/medulloblastoma. Eur J Cancer. 2002;38(1):83–91.

17. Pfister S, Schlaeger C, Mendrzyk F, Wittmann A, Benner A, Kulozik A, et al. Array-based profiling of reference-independent methylation status (aPRIMES) identifies frequent promoter methylation and consecutive downregulation of ZIC2 in pediatric medulloblastoma. Nucleic Acids Res. 2007;35(7).

18. Nakahara Y, Northcott PA, Li M, Kongkham PN, Smith C, Yan H, et al. Genetic and epigenetic inactivation of Kruppel-like Factor 4 in medulloblastoma. Neoplasia. 2010;12(1):20–7.

19. Diede SJ, Guenthoer J, Geng LN, Mahoney SE, Marotta M, Olson JM, et al. DNA methylation of developmental genes in pediatric medulloblastomas identified by denaturation analysis of methylation differences. Proc Natl Acad Sci U S A. 2010;107(1):234–9.

20. Kongkham PN, Northcott PA, Ra YS, Nakahara Y, Mainprize TG, Croul SE, et al. An epigenetic genome-wide screen identifies SPINT2 as a novel tumor suppressor gene in pediatric medulloblastoma. Cancer Res. 2008;68(23):9945–53.

21. Lindsey JC, Kawauchi D, Schwalbe EC, Solecki DJ, Selby MP, Mckinnon PJ, et al. Cross-species epigenetics identifies a critical role for VAV1 in SHH subgroup medulloblastoma maintenance. Oncogene. 2015;34:4746–57.

22. Hovestadt V, Jones DTW, Picelli S, Wang W, Kool M, Northcott PA, et al. Decoding the regulatory landscape of medulloblastoma using DNA methylation sequencing. Nature. 2014 Jun 18;510(7506):537–41.

23. Sadakierska-Chudy A, Filip M. A Comprehensive View of the Epigenetic Landscape. Part II: Histone Post-translational Modification, Nucleosome Level, and Chromatin Regulation by ncRNAs. Neurotox Res. 2015; 27(2): 172–197

24. Yi J, Wu J. Epigenetic regulation in medulloblastoma. Mol Cell Neurosci. 2018 Mar; 87: 65–76.

25. Roussel MF, Stripay JL. Epigenetic Drivers in Pediatric Medulloblastoma. Cerebellum. 2018; 17(1): 28–36.

26. Pfister S, Rea S, Taipale M, Mendrzyk F, Straub B, Ittrich C, et al. The histone acetyltransferase hMOF is frequently downregulated in primary breast carcinoma and medulloblastoma and constitutes a biomarker for clinical outcome in medulloblastoma. Int J Cancer. 2008;122(6):1207–13.

27. Milde T, Oehme I, Korshunov A, Kopp-Schneider A, Remke M, Northcott P, et al. HDAC5 and HDAC9 in medulloblastoma: Novel markers for risk stratification and role in tumor cell growth. Clin Cancer Res. 2010;16(12):3240–52.

28. Robinson G, Parker M, Kranenburg TA, Lu C, Chen X, Ding L, et al. Novel mutations target distinct subgroups of medulloblastoma. Nature. 2012;488(7409):43–8.

29. Shi J, Vakoc CR. The Mechanisms behind the Therapeutic Activity of BET Bromodomain Inhibition. Mol Cell. 2014;54(5):728–36.

30. Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, et al. Role of histone H3 lysine 27 methylation in polycomb-group silencing. Science. 2002;298(5595):1039–43.

31. Dubuc AM, Remke M, Korshunov A, Northcott PA, Zhan SH, Mendez-Lago M, et al. Aberrant patterns of H3K4 and H3K27 histone lysine methylation occur across subgroups in medulloblastoma. Acta Neuropathol. 2013;125(3):373–84.

32. Clapier CR, Iwasa J, Cairns BR, Peterson CL. Mechanisms of action and regulation of ATP-dependent chromatin-remodelling complexes. Nat Rev Mol Cell Biol. 2017 Jul; 18(7): 407–422.

33. Shi X, Wang Q, Gu J, Xuan Z, Wu JI. SMARCA4/Brg1 coordinates genetic and epigenetic networks underlying Shh-type medulloblastoma development. Nat Publ Gr. 2016;35:5746–58.

34. Leung JWC, Makharashvili N, Agarwal P, Chiu LY, Pourpre R, Cammarata MB, et al. ZMYM3 regulates BRCA1 localization at damaged chromatin to promote DNA repair. Genes Dev. 2017;31(3):260–74.

35. Motameny S, Wolters S, Nürnberg P, Schumacher B. Next Generation Sequencing of miRNAs – Strategies, Resources and Methods. Genes. 2010;1:70–84.

36. Gokhale A, Kunder R, Goel A, Sarin R, Moiyadi A, Shenoy A, et al. Distinctive microRNA signature of medulloblastomas associated with the WNT signaling pathway. J Cancer Res Ther. 2010;6(4):521–9.

37. Northcott PA, Fernandez-L A, Hagan JP, Ellison DW, Grajkowska W, Gillespie Y, et al. The miR-17/92 polycistron is up-regulated in sonic hedgehog-driven medulloblastomas and induced by N-myc in sonic hedgehog-treated cerebellar neural precursors. Cancer Res. 2009;69(8):3249–55.

38. Weeraratne SD, Amani V, Teider N, Pierre-Francois J, Winter D, Kye MJ, et al. Pleiotropic effects of miR-183~96~182 converge to regulate cell survival, proliferation and migration in medulloblastoma. Acta Neuropathol. 2012;123(4):539–52.

39. Grunder E, D'ambrosio R, Fiaschetti G, Abela L, Arcaro A, Zuzak T, et al. MicroRNA-21 suppression impedes medulloblastoma cell migration. Eur J Cancer. 2011;47(16):2479-90.

40. Pal R, Greene S. microRNA-10b Is Overexpressed and Critical for Cell Survival and Proliferation in Medulloblastoma. PLoS One. 2015;10(9):e0137845.

41. Li KK-W, Xia T, Ma FMT, Zhang R, Mao Y, Wang Y, et al. miR-106b is overexpressed in medulloblastomas and interacts directly with PTEN. Neuropathol Appl Neurobiol. 2015;41(2):145–64.

42. Pierson J, Hostager B, Fan R, Vibhakar R. Regulation of cyclin dependent kinase 6 by microRNA 124 in medulloblastoma. J Neurooncol. 2008;90(1):1–7.

43. Ferretti E, De Smaele E, Miele E, Laneve P, Po A, Pelloni M, et al. Concerted microRNA control of Hedgehog signalling in cerebellar neuronal progenitor and tumour cells. EMBO J. 2008;27(19):2616–27.

44. Joshi P, Katsushima K, Zhou R, Meoded A, Stapleton S, Jallo G, et al. The therapeutic and diagnostic potential of regulatory noncoding RNAs in medulloblastoma. Neuro-Oncology Adv. 2019;1(1):1–14.

45. Iyer MK, Niknafs YS, Malik R, Singhal U, Sahu A, Hosono Y, et al. The landscape of long noncoding RNAs in the human transcriptome. Nat Genet. 2015;47(3):199–208.

46. Laneve P, Po A, Favia A, Legnini I, Alfano V, Rea J, et al. The long noncoding RNA linc-NeD125 controls the expression of medulloblastoma driver genes by microRNA sponge activity. Oncotarget. 2017;8(19):31003-31015.

47. Zhang Y, Wang T, Wang S, Xiong Y, Zhang R, Zhang X, et al. Nkx2-2as suppression contributes to the pathogenesis of sonic hedgehog medulloblastoma. Cancer Res. 2018;78(4):962–73.

48. Kesherwani V, Shukla M, Coulter DW, Sharp JG, Joshi SS, Chaturvedi NK, et al. Long non-coding RNA profiling of pediatric Medulloblastoma. BMC Med Genomics. 2020;13(1):1–14.