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Molecular Approach to Neuroblastoma

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Abstract

Neuroblastoma is a notably malignant cancer originates from neuroblastoma stem cells during embryogenesis. It can originate from any region of the peripheral nervous system. Neuroblastoma is a heterogeneous cancer. The cells responsible for heterogeneous structure are neuroblastoma stem cells that initiate the cancer and generate into all the cancer cells and have self-renewal property. Although some specific surface markers and genetic patterns of neuroblastoma stem cell were determined, all mechanisms have not been illuminated yet. Mutations that are specific to neuroblastoma development, risk group, and disease-stage are identified. However, epigenetic dysregulations also play major roles in the development of neuroblastoma. Patients gradually develop resistance to conventional chemotherapy or relapse occurs after treatment. New therapy approaches have been developed, either as alternatives to conventional chemotherapy, or in combination with it, in order to overcome the handicaps. Targeted therapies, those directly affecting the cancer cell or the cancer stem cell and having a minimal effect on healthy cells, constitute these approaches. Since neuroblastoma is highly heterogeneous both genetically and epigenetically, the data obtained from molecular mechanisms will greatly contribute to the survival of patients.

Keywords: neuroblastoma, neuroblastoma stem cell, molecular, epigenetic, miRNA, lncRNA, targeted therapy

1. Introduction

Neuroblastoma is a type of neural crest originated cancer, can arise in any region of the sympathetic nervous system, but occurs in more than 50% of adrenal glands. Metastasis usually occurs to the lymph nodes, bone marrow, bones and rarely to the lung, liver, and central nervous system. Metastasis is the most important factor that decreases survival rates to 40%. Clinical symptoms can vary according to the primary tumor site and the site of metastasis.

Incidence and phenotype of the disease vary by the age, sex, and ethnicities; however, neuroblastoma is regarded as an orphan disease, with approximately eight cases per million in the worldwide and these cases comprise 7% of all childhood cancers. The maximal rate of the cases occurs in the perinatal age. The median age of the neuroblastoma is 18 months and 90% of the patients are under 10 years of age. When seen in adolescents who constitute 5% of all cases, usually exhibit chemotherapy-resistant character.

With the International Neuroblastoma Pathology Committee (INPC) modification of the Shimada classification of 1984, the neuroblastoma was divided into four categories according to morphological and biological characteristics of the cells, and patient age: neuroblastoma, intermixed ganglioneuroblastoma, ganglioneuroma, and nodular ganglioneuroblastoma. Although many different staging systems have been used in the past, International Neuroblastoma Staging System (INSS), including 1, 2A, 2B, 3, 4 and 4S stages, which are formed according to tumor size and metastasis status, is currently used. By combining the disease stages with prognostic factors, very low, low, medium, high-risk groups were formed.

The spontaneous regression has been observed for patients with stage 4S who have limited metastatic characteristics below 1 year of age. Neuroblastoma is less common but more malignant in people with African ancestry than European ones. Moreover, the disease is infrequent in girls than in boys. However, genetic, epigenetic, and environmental factors, which affect the incidence, have not been openly described yet.

Although neuroblastoma is initially considered a familial disease, it is now known that familial cases constitute only 1% of all cases. In particular, germline gain-of-function mutations of the anaplastic lymphoma receptor tyrosine kinase (ALK) and paired like homeobox 2b (PHOX2B) genes are responsible for these familial events, while neuroblastoma has been described as a complex disease resulting from combination of many different allelic effects.

Neuroblastoma is originated from the neuroblastoma stem cell, which is the resultant genetic alterations of progenitor cells that will differentiate into the sympathetic nervous system. The amplification of the V-Myc Avian Myelocytomatosis Viral Oncogene Neuroblastoma-Derived Homolog (MYCN) gene, a transcriptional regulator that promotes cell cycle and differentiation, induces activation of oncogenes and inhibition of tumor suppressors, is the most common genetic alteration in neuroblastoma. MYCN amplification has been associated with tumor grade, progression, and metastasis. ALK mutations also play a role in neuroblastoma pathology, leading to the activation of oncogenic signaling pathways by somatic amplification as well as familial predisposition. Polymorphisms and overexpression of the Lin-28 Homolog B (LIN28B) gene, involved in the regulation of multiple signaling pathways, play a role in neuroblastoma formation and progression more often than amplification. Loss-of-function mutations in the transcriptional regulator Chromatin Remodeler (ATRX) gene, regulation in the promoter region of the telomerase enzyme Telomerase Reverse Transcriptase (TERT) gene, mutations in genes involved in chromatin remodeling are associated with neuroblastoma development and progression. Somatic mutations in noncoding regions as much as in coding regions have also been associated with neuroblastoma progression. In particular, mutations in noncoding regions of tumor suppressor and candidate tumor suppressor genes are highly effective on high-risk neuroblastoma.

Laboratory tests, radiographic imaging, and histological staining methods are used in the diagnosis of neuroblastoma, staging, and monitoring the treatment. The increased levels of catecholamine and its metabolites in urine are the most commonly used biomarkers in neuroblastoma. More rarely, increased levels of adrenaline derivatives are found in the plasma. Radiological approaches as ultrasonography, CT, and MRI are used for staging and metastatic profiling. Molecular analysis of genes such as MYCN, ALK is also important in diagnosis and routine monitoring. For this purpose, mutations in biopsy specimens can be detected at single cell level using Fluorescence In Situ Hybridization (FISH), real-time Polymerase Chain Reaction (PCR), flow cytometry, Single-nucleotide polymorphism (SNP) arrays, Next-Generation Sequencing (NGS), and microarray methods.

Neuroblastoma treatment includes surgical removal of the primer tumor, standard chemotherapy, and induction chemotherapy, autologous hematopoietic stem cell transplantation following by myeloablative chemotherapy and radiotherapy approaches, depending on the risk group of the patients. Unfortunately, over time, resistance to chemotherapeutics occurs in the patient and relapses frequently occur after the treatment. To overcome these handicaps, research for neuroblastoma treatment has been focused on targeted therapy strategies to improve survival of patients.

2. Molecular pathology of neuroblastoma

The most common genetic and epigenetic alterations in neuroblastoma include MYCN, ALK, PHOX2B, ATRX, TERT, Tumor Protein P53 (TP53), Lysine methyltransferases (KMTs), Histone lysine demethylases (KDMs), and Histone deacetylase (HDAC) genes, noncoding RNA (ncRNA) expression changes [1]. Although only a few mutations that define neuroblastoma development, prognosis, and metastatic characteristics have been identified, many of the genetic alterations underlying this rare disease remain to be discovered yet to improve treatment success and patient survival. With the development of novel methods in the molecular medicine in recent years, the studies in this area and the resulting findings are rapidly increasing and allow for the development of promising approaches in the treatment.

2.1. Neuroblastoma-specific genetic alterations

Amplification of the MYCN proto-oncogene has been the first discovered aberration associated with neuroblastoma pathogenesis. In a study realized by Brodeur et al. in 1984, it was determined that the DNA copy number of the MYCN gene in the human neuroblastoma cell line is 20–140 times amplified, unlike other human cancer cell lines. In accordance with the result, they showed that the copy number of MYCN is correlated with the disease stage and poor prognosis in untreated patients [2].

The MYCN proto-oncogene, also referred to as N-myc, localized in 2p24.3, is a transcription factor that is homologous to the MYC (c-MYC) proto-oncogene localized in 8q24.21, whose mutations have been associated with hematological malignancies and lymphomas [3, 4]. MYCN alterations have been related with various solid tumors, especially neuroblastoma. It

is localized in the nucleus and activates transcription of many genes that support cell survival and proliferation via dimerization with the other transcription factors, which have the same binding domain. It also suppresses the genes responsible for normal cell differentiation [5]. Accumulation of neuroblasts with the impairment of normal differentiation of neuronal cells is the underlying cause of primer neuroblastoma formation.

Familial neuroblastomas rarely occur and constitute approximately 1–2% of all cases. The tyrosine kinase receptor ALK, which plays an essential role in the development of the normal brain and nervous system, is the major component of hereditary neuroblastomas [6]. However, abnormal ALK expression plays an important role not only in hereditary neuroblastoma but also in the development of sporadic neuroblastoma [7].

In addition to ALK, PHOX2B gene, the main regulator of neural crest development is responsible for the development of familial neuroblastoma [8].

While MYCN amplification is responsible for a large proportion of sporadic neuroblastomas (~20%), a large proportion of adolescent and young adult patients have ATRX mutations (~20%) without MYCN amplification [9]. ATRX, a chromatin remodeling gene that is a member of the SWI/SNF family, plays a role in the regulation of gene expression by the epigenetic mechanism by organizing the matrix-chromatin interaction [10]. ATRX is responsible for H3.3 accumulation in methylation silencing regions such as transposon elements, imprinted genes, and telomeres [11]. Telomere-repeat sequences are located at the end regions of the chromosomes and maintain the stability of the chromosomes. In eukaryotic cells, telomeres are shortened at each replication, thus limiting the proliferation ability of the cell [12]. There are two mechanisms involved in maintaining the telomere length, which leads to the proliferation capacity of both stem/progenitor cells and cancer cells.

First, these are the activation of the alternative lengthening of telomeres (ALT) mechanism, which involves the loss of function of the ATRX gene. Mutations of the ATRX gene cause abnormally long telomere lengths in cancers, including neuroblastoma [13].

The second mechanism that plays a role in the conservation of telomere length is the activation of the telomerase enzyme by rearrangement of the TERT gene. Mutations in the TERT gene are highly associated with high-stage neuroblastoma patients (~20%) who do not have MYCN and ATRX mutations and are associated with poor prognosis [14].

The catastrophic process, called chromothripsis, describes a new carcinogenesis mechanism that is caused by a large number (tens to hundreds) of rearrangements occurring in the same cell in one or several chromosomes, unlike the conventional mechanism in which the accumulation of mutations over time causes cancer [15]. Chromothripsis mechanisms have been highly defined through recent whole-genome sequencing studies. Defects that play a role in the mechanism occur in genes that are involved in nervous system development and neurogenesis. Defects of the transmembrane protein tyrosine phosphatase, receptor type D (PTPRD), teneurin transmembrane protein 2 (TENM2), teneurin transmembrane protein 3 (TENM3), CUB and sushi multiple domains 1 (CSMD1) proteins which play a role in nervous system development and T-cell lymphoma invasion and metastasis 1 (TIAM1), Rho GTPase activating protein (DLC1) GTPase proteins involved in Rac/Rho signaling through these

transmembrane proteins are responsible for the development of high-grade neuroblastoma without MYCN amplification. Down regulation of the cell division cycle 42 (CDC42) gene, a GTPase located in 1p36, is characterized in advanced disease patients with MYCN amplification [16].

In addition to CDC42, 1p36 deletion including RhoGEF kinase (KALRN), calmodulin binding transcription activator 1 (CAMTA1), kinesin family member 1B (KIF1B), castor zinc finger 1 (CASZ1) genes and 17q acquisition are correlated with MYCN amplification in the high-stage neuroblastoma [17, 18]. 11q loss of heterozygosity that is inversely related to MYCN amplification is also responsible for a large proportion of high-stage neuroblastoma.

Large spectrum of genetic-wide association studies has identified many genetic alterations related to predisposition to nonfamilial (sporadic) neuroblastomas, recently. The genes associated with genetic predisposition are BRCA1-associated RING domain 1 (BARD1), which regulates tumor suppressor BRCA1 activity, transcriptional regulator LIM domain only 1 (LMO1), dual specificity phosphatase 12 (DUSP12) that controls cell proliferation, DEAD-box helicase 4 (DDX4), a helicase that regulates the secondary structure of RNA and its associated functions, interleukin 31 receptor A (IL31RA), hydroxysteroid 17-beta dehydrogenase 12 (HSD17B12) which plays role in fatty acid biosynthesis, HECT Domain and ankyrin repeat containing E3 ubiquitin protein ligase 1 (HACE1) that regulates proteosomal degradation, LIN28B, neurofilament light (NEFL) that plays a role in the formation of neurons and TP53 gene, one of the most important transcription factor and a tumor suppressor that regulates essential cellular events as apoptosis, DNA repair [19–21].

2.2. Epigenetic pattern in neuroblastoma

Epigenetic modifications are reversible changes that play a role in the regulation of gene expression by regulating the chromatin accessibility of the elements necessary for transcription in eukaryotic cells via chromatin remodeling, histone modifications, DNA methylation, and noncoding RNAs. While acetylation is only associated with euchromatin, methylation regulates both euchromatin and heterochromatin [22].

Results from microarray-based DNA methylation studies performed in neuroblastoma patients have shown that gene-specific (promoter) hypomethylation occurs more frequently than genomic hypermethylation that cause development of neuroblastoma [23, 24]. Both upregulation and downregulation of the DNA methyltransferases may be associated with the pathogenesis of neuroblastoma, depending on the functions of the genes which have promoter methylation. O-6-methylguanine-DNA methyltransferase (MGMT), a DNA methyltransferase, interacting with the Wnt/B-catenin signaling pathway is upregulated in the neuroblastoma and is associated with chemotherapy resistance [25]. However, DNA methyltransferase 3 beta (DNMT3B7), a DNA methyltransferase, regulates genomic methylation in neuroblastoma and triggers normal neuronal differentiation [26].

Similar to DNA methylation, histone methylation and demethylation have different effects on neuroblastoma prognosis. Lysine methyltransferase 5A (KMT5A), a H4K20me1 methyltransferase, promotes survival and differentiation in neuroblastoma cells by suppressing p53 mediated

apoptosis [27]. Overexpression of the DOT1-like histone lysine methyltransferase (DOT1L) histone methyltransferase correlates with expression levels of MYCN, solute carrier family 6 member 4 (SLC6A4), and E2F transcription factor 2 (E2F2) genes, triggers the development of neuroblastoma and has been associated with poor prognosis [28]. Histone chaperone chromatin assembly factor 1 subunit A (CHAF1A) promotes advanced-stage neuroblastoma development via H3K9 trimethylation of the survival genes [29]. Lysine demethylase 4B (KDM4B), a lysine demethylase, is responsible for epigenetic regulation of MYCN signaling via histone demethylation in poor prognosis of neuroblastoma [30].

Since the identification of their roles in the pathogenesis of many solid and hematological malignancies, a large proportion of treatment strategies have been targeting epigenetic mechanisms. Because histone acetylation is common in cancer prognosis, investigations focus on HDAC inhibitors, especially. Histone deacetylase 2 (HDAC2) contributes neuroblastoma progression through downregulation of apoptotic miR-183 signaling [31]. The grainyhead-like transcription factor 1 (GRHL1) is suppressed by the promoter hypoacetylation via histone deacetylase 3 (HDAC3) in the advanced-level neuroblastoma [32].

Suppression of CD9 expression in the neuroblastoma by transcriptional activity of histone deacetylase 5 (HDAC5) has been associated with poor prognosis and metastasis [33]. Increased levels of histone deacetylase 8 (HDAC8) expression in neuroblastoma cells have been suggested to play a role in resistance to chemotherapeutics by suppressing the expression of miR-137 and triggering the expression of the ATP binding cassette subfamily B member 1 (MDR1) gene [34]. Upregulation of the SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily A, member 4 (SMARCA4), a chromatin remodeling gene is in the same family with the ATRX, promotes viability of the neuroblastoma cells and it is related to advanced-stage neuroblastoma [35].

As the result of the outputs of the ENCODE project, which was published in 2012, less than 2% of the human genome included protein-coding genes, and large parts of transcriptome composed of pseudogenes and ncRNAs. ncRNAs, which play essential roles both in the regulation of gene expression and in the protein synthesis, contain small RNAs (tRNA, microRNA(miRNA), siRNA, snRNA) and long noncoding RNAs (lnc RNA). miRNAs, single-stranded RNA molecules of about 20 nucleotides and lncRNAs, longer than 200 nucleotides are essential molecules in the gene expression regulation. Since ncRNAs have critical regulatory roles, deregulations are associated with multiplexed pathologies, especially cancers [36].

miRNAs are the basic epigenetic molecules involved in all stages of gene expression regulation. Gene expression has oncogenic (oncomiR) or tumor suppressor properties depending on their regulatory properties.

Members of the miR-17-92 cluster (miR-17-5p, miR-18a, miR-19a, miR-20a, and miR-92) are upregulated with MYCN amplification indicating poor prognosis and treatment resistance via regulation of a main cell cycle regulator cyclin dependent kinase inhibitor 1A (P21) and a apoptotic regulator BCL2-like 11 (BIM) proteins in neuroblastoma cell lines

and patients [37, 38]. miR-34a, which is associated with TP53 signaling pathway, acts as a tumor suppressor and downregulates MYCN, E2F transcription factor 3 (E2F3), apoptosis regulator (BCL2), a cell cycle component cyclin D1 (CCND1), and CDK expression. However, in neuroblastoma cells, miR-34a expression is generally silenced with 1p36 deletion [17]. miR-497 is also a tumor suppressor that similarly suppresses the MYCN expression [39]. Upregulation of miR-188-5p and miR-501-5p and downregulation of miR-125b-1 are thought to be associated with chemotherapy resistance [40]. The expression increase of oncomiR miR-221, a negative regulator of the nemo-like kinase (NLK) gene, is characterized by tumor progression and poor prognosis in neuroblastoma cells in relation to MYCN [41]. The low expression of dicer 1, ribonuclease III (DICER), and drosha ribonuclease III (DROSHA) genes, which play a role in the miRNA biogenesis, generally leads to a decrease of miRNA expression in neuroblastoma [42].

Since 2012, lncRNAs have begun to become part of neuroblastoma research, like as the other cancer researches. Studies in this area have focused particularly on the regulation of the expression of genes such as MYCN, ALK, which are highly associated with neuroblastoma pathogenesis, and directing the neuroblastoma cells to apoptotic death. In particular, biomarkers have been identified that indicate a poor prognosis and a high-risk disease group. A cell proliferation regulator Mir-100-Let-7a-2 cluster host gene (MIR100HG) promotes neuroblastoma cell proliferation [43]. lncRNA metastasis associated lung adenocarcinoma transcript 1 (MALAT1) regulates the genes which are responsible for neuronal differentiation, angiogenesis, and migration in neuroblastoma [44–46]. While MYCN upstream transcript (MYCNUT) and small nucleolar RNA host gene 1 (SNHG1) which are associated with high risk, trigger neuroblastoma progression via MYCN amplification, cyclin dependent kinase inhibitor 2A (CAI2), and long intergenic nonprotein coding RNA 467 (LINC00467) promote the neuroblastoma independently of the MYCN expression [47–50]. Downregulation or loss of tumor suppressor lncRNAs cancer susceptibility candidate 15 (CASC15-S) and neuroblastoma associated transcript 1 (NBAT-1), and upregulation of onco-lncRNA ncRAN are associated with advanced-stage neuroblastoma and poor prognosis via increasing cell proliferation and disregulating neuronal differentiation [51–53].

Anti-apoptotic lncRNAs growth arrest specific 5 (GAS5), long intergenic nonprotein coding RNA 1105 (LINC01105) are located in TP53 signaling pathway and regulate apoptosis in neuroblastoma cells. Activation of the lncRNA GAS5, which has two alternative transcripts that both suppress TP53, DNA repair associated (BRCA1) and growth arrest and DNA damage inducible alpha (GADD45A) and stabilize proto-oncogene, E3 ubiquitin protein ligase (MDM2), promotes tumor proliferation in neuroblastoma cells by inhibiting apoptosis and cell cycle arrest [54]. LINC01105 upregulation also suppresses TP53-related apoptosis in neuroblastoma cells. On the other hand, proapoptotic lncRNA maternally expressed 3 (MEG3) is also located TP53 signal pathway and downregulation of MEG3 causes suppression of apoptosis and deregulated differentiation in the neuroblastoma tissues [55]. Even though the epigenetic changes may regulate the prognosis and progression stage in neuroblastoma patients, the knowledge about the regulation mechanisms is still inadequate (**Figure 1**).

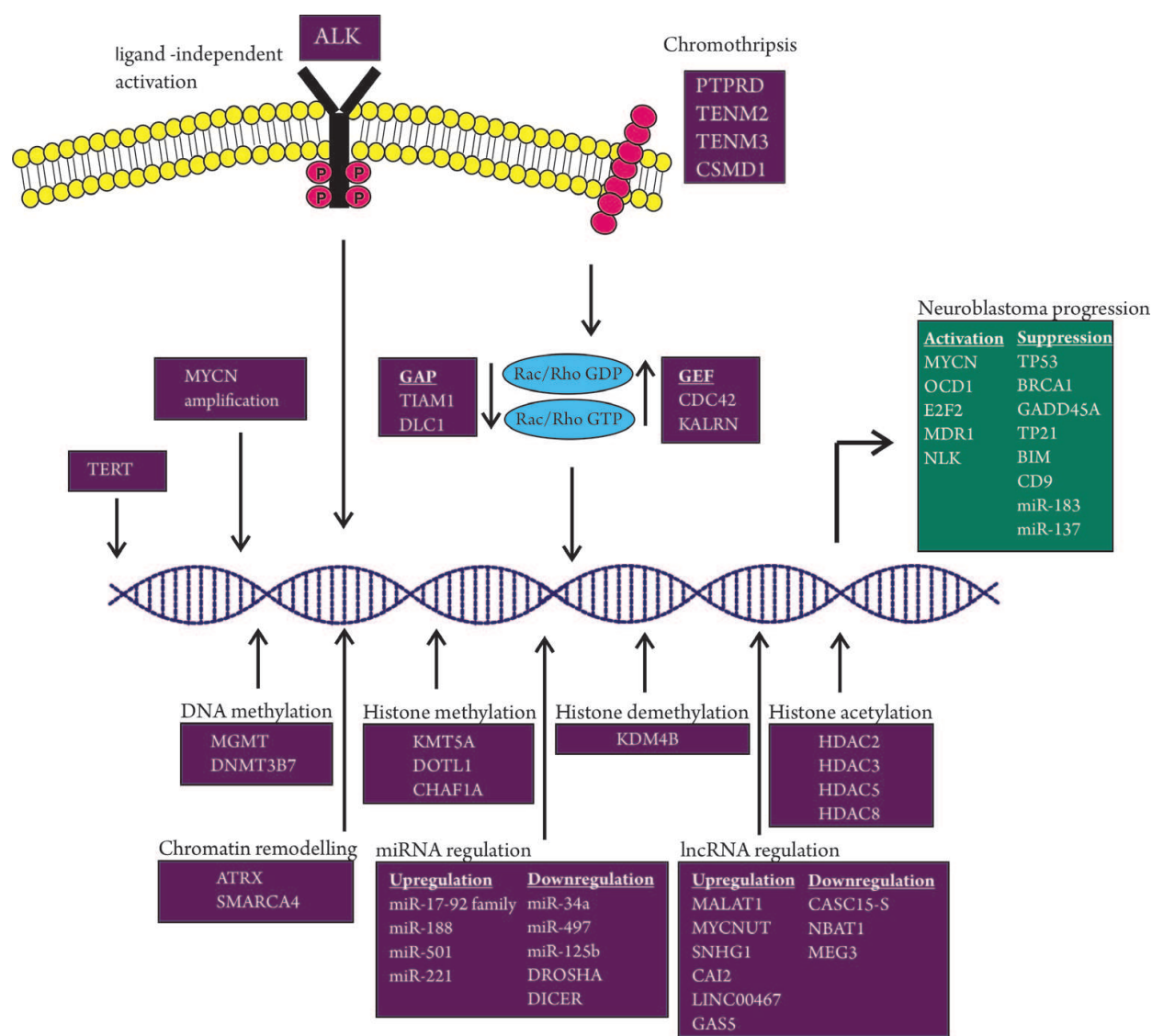


Figure 1. Genetic and epigenetic mechanisms in neuroblastoma.

3. Neuroblastoma stem cell

Neuroblastoma is a heterogeneous cancer and there are many different specific cells in a single tumor. It has been suggested that all types of cancer cells that provide heterogeneous properties are characterized by the differentiation of a single neuroblastoma stem cell [56]. Because cancer stem cells are closely related to both chemotherapy resistance and relapse, the elucidation of the molecular mechanisms of neuroblastoma stem cells is crucial for treatment success.

Neuroblastoma stem cells were first described as I-type cells (intermediate type), malignant cells of neural crest, morphologically located between neuroblastic cells and neural crest cells. I cells are characterized as stem cells because they can produce self-renewal cell lines of two cell types [57].

Polarity loss and asymmetric division of neuronal cells constitute neuroblastoma stem cells during normal neuronal development have been shown in studies conducted in *Drosophila melanogaster* [58]. Speedy/RINGO cell cycle regulator family member A (SPDYA) regulates the formation of neuroblastoma stem cells by controlling asymmetric division of cells [59]. ALK and MYCN mutations, which have the most important share in the progression of familial and sporadic neuroblastomas, respectively, can constitute neuroblastoma stem cells from neural crest progenitor cells [60]. Repression of TP53 by proto-oncogene, polycomb ring finger (BMI1) causes neuroblastoma induction from embryonic precursors by reducing the response to oncogenic transformation [61]. It is suggested that polo-like kinase 1 (PLK1) expression in neuroblastoma stem cells is one of the factors that contribute to survival and self-renewal [62].

Neuroblastoma stem cells are characterized by upregulation of prominin1 (PROM1/CD133), proto-oncogene receptor tyrosine kinase (KIT/CD117), colony stimulating factor 3 receptor (CSF3R/CD114) cell surface proteins and G protein-coupled receptor class C group 5 member C (GPRC5C), NOTCH1, placental growth factor (PGF), neurotrophic receptor tyrosine kinase 2 (NTRK2), nerve growth factor receptor (NGFR), colony stimulating factor 3 (CSF3), signal transducer and activator of transcription 3 (STAT3), and RB transcriptional corepressor-like 2 (RBL2) genes which play a role in differentiation to malignant neuroblastoma stem cell (Figure 2) [63–66].

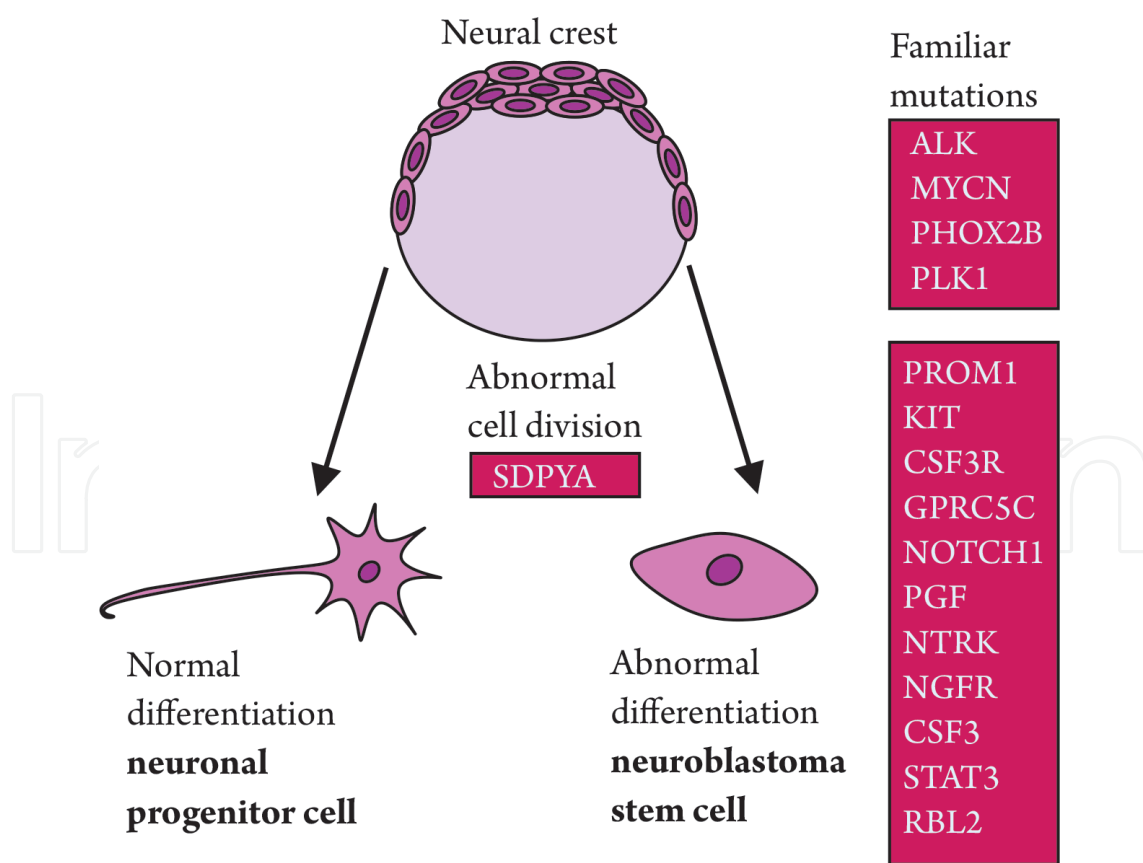


Figure 2. Neuroblastoma stem cell.

4. Targeted treatment approach and future perspectives

Retinoic acid, platinum complexes, DNA alkylating agents, and topoisomerase inhibitors are used in the conventional chemotherapy of neuroblastoma. Nevertheless, neuroblastoma cells become resistant to the chemotherapeutics during the time. Mechanisms that cause chemotherapy resistance are frequently associated with MYCN. A novel retinoic acid resistance mechanism includes LIM domain only 4 (LMO4), cytochrome P450 family 26 subfamily A member 1 (CYP26A1), achaete-scute family BHLH transcription factor 1 (ASCL1), ret proto-oncogene (RET), frizzled class receptor 7 (FZD7), and dickkopf WNT signaling pathway inhibitor 1 (DKK1) genes are triggered by MYCN overexpression. These causes may lead to targeting of TGF- β signaling pathway, leading to resistance [67]. MYCN plays a different critical role in resistance to platinum compounds by inhibiting apoptosis through deregulation of PPARG coactivator 1 alpha (PPARGC1A), transcription factor A, mitochondrial (TFAM) genes regulating mitochondrial biogenesis and mitochondrial dynamin-like GTPase (OPA1), mitofusin 2 (MFN2), dynamin 1-like (DRP1) genes regulating mitochondrial dynamics [68]. The identification of resistance mechanisms taking place in different pathways is still ongoing. Most recently, two different resistance formation has been described both calcium metabolism and activation of the hepatocyte growth factor (HGF)/hepatocyte growth factor receptor (MET) signaling pathway [69, 70]. Resistant-dependent or independent relapse also limits the success of conventional chemotherapy.

Conventional chemotherapy resistance and relapse risk have led research to focus on targeted therapy. Currently, targeted treatment approaches aim to induce apoptosis of neuroblastoma cells, dominantly. Moreover, studies of the induction of normal neuronal differentiation, epigenetic regulation, immunotherapy, nanoparticles, and dual mechanisms have been the subject of recent research.

Upregulation of PLK1, the positive regulator of cell cycle and MYCN stabilization, has been associated with high-risk neuroblastoma. Inhibition of PLK1 causes cell cycle arrest and induces apoptosis [71, 72]. Unlike many cancers, TP53 mutations occur less frequently in neuroblastoma. The inhibition of protein phosphatase, Mg^{2+}/Mn^{2+} dependent 1D (PPM1D/Wip1), which is a negative regulator of TP53 mediated cell-death pathway, has been proposed as a novel approach to induce apoptosis through neuroblastoma cells through checkpoint kinase 2 (CHK2)/TP53 [73]. The novel identified proapoptotic brain expressed X-linked (BEX) genes in the downstream of the TP53 signaling pathway are promising as new tumor suppressors by inducing apoptosis in neuroblastoma cells [74]. Inhibition of epidermal growth factor receptor (EGFR) directs neuroblastoma cells to apoptosis through the suppression of the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/serine/threonine kinase (AKT)/mechanistic target of rapamycin (mTOR) signaling pathway [75]. The inhibition of the G-protein associated receptor tachykinin receptor 1 (TACR1) induces apoptosis and reduces survival in neuroblastoma cells [76]. Targeting the proto-oncogene, nonreceptor tyrosine kinase (SRC)/proto-oncogene 1, nonreceptor tyrosine kinase (ABL) presents a new therapeutic approach by directing neuroblastoma cells to death [77]. Interleukin 24 (IL-24) induces cell death in neuroblastoma via caspase-independent pathway via apoptosis inducing factor (AIF), serine/threonine kinase (ATM), and H2A histone family member X (H2AFX) regulation [78].

Another approach is the induction of normal differentiation of neuroblastoma cells. The ASCL1 gene prevents the neuronal differentiation of neuroblast cells, the main cause of neuroblastoma development, through a mechanism independent of MYCN oncogenes [79]. Coactivation of PPARG regulates cell differentiation, and retinoic acid receptor alpha (RARA), which is involved in conventional therapy, composes a new combinational approach to neuroblastoma therapy by inducing normal differentiation of neuroblasts [80]. Coinhibition of ALK and CDK4/6, Anti-GD2 mAb and HDAC, aurora kinase A (AURKA), and BCL-2, also constitute synergistic approaches to neuroblastoma treatment [81–83].

Epigenetic regulation has great importance in the treatment as well as in the pathogenesis of neuroblastoma. Inhibition of HDAC11 in neuroblastoma suppresses genes associated with proliferation and induces apoptosis. HDAC11 can be seen as a promising goal for treatment [84]. HDAC8 inhibition and miR-137 expression lead to increased chemotherapy sensitivity [34]. miR-497, which regulates the genes associated with proliferation, metastasis, and resistance, is a novel candidate molecule for targeted neuroblastoma therapy [85]. The epigenetic regulator miR-506 suppresses the metastasis of the neuroblastoma cells via inhibiting Rho associated coiled-coil containing protein kinase 1 (ROCK1) which is located in transforming growth factor beta (TGF- β) signaling pathway [86]. Upregulation of ncRNA 45A plays a critical role in tumor proliferation and metastasis by regulating the expression of amyloid beta precursor protein binding family B member 2 (FE65L1), G2, and S-phase expressed 1 (GTSE1) genes [87].

Digestive organ expansion factor (DEF) plays a role in ribosome biogenesis, acts as a regulator in both the development of the peripheral sympathetic nervous system and the development of neuroblastoma. DEF and the other components of the small ribosomal subunit processing involved in ribosome biogenesis have potential use in neuroblastoma targeted therapy [88]. Human ion channel transient receptor potential cation channel subfamily M member 2 (TRPM2), which regulates cell proliferation via mitochondria, is a potential therapeutic target in neuroblastoma [89]. One of the promising approaches in neuroblastoma therapy is to target cell surface proteins such as solute carrier family 6 member 2 (HNET) regulates neurotransmitter homeostasis, ALK, and NTRK2 and neural cell adhesion molecule (NCAM) [90, 91].

It is a promising approach to vaccination through chimeric antigen receptor (CAR)-modified T cells both to create an immunological response to neuroblastoma cells and to increase the level of response [92]. Cancer/testis antigen 1B (CTAG1B) is expressed by various solid tumors including the neuroblastoma, a potential immunotherapy target [93].

Nanoparticles have great potential for the use of diagnosis through fluorescent probes and treatment via encapsulated therapeutic genes (siMyc, siBcl-2, and siVEGF) of neuroblastoma [94]. Targeting drug delivery to neuroblastoma cells may be achieved by genetically engineered biological nanoporous molecules such as diatoms so minimizing damage to healthy cells [95].

Clustered regularly interspaced short palindromic repeats (CRISPRs) and CRISPR-associated protein-9 nuclease (Cas9) systems are valuable targeted genome editing tools that have great potential for molecular medicine applications. The studies aimed to clarify the mechanisms that play a role in pathogenesis and to develop targeted therapies. The activity of aldehyde dehydrogenase 1 (ALDH1) isoenzymes was associated with the aggressive nature of neuroblastoma stem cells in the study using patient-derived xenograft tumors using CRISPR/Cas9

technology [96]. In animal models, DNA methyltransferase 3 alpha (DNMT3a) transactivation using CRISPR/Cas9 technology contributes to the regulation of the methylation of brain cells [97]. Using CRISPR/Cas9 technology, silencing mutant neuroblastoma RAS viral oncogene homolog (NRAS) gene through guide RNAs (gRNAs) in the NRAS-mutant cell line has made cells more sensitive to specific inhibitors [98].

Increased knowledge of neuroblastoma pathology and molecular biology will contribute to the creation of new approaches to diagnosis and treatment and to increased patient life quality and survival rates.

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