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REVIEW ARTICLE

The roles played by the MYCN, Trk, and ALK genes in neuroblastoma and neural development

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Abstract
Neuroblastoma is one of the most frequent, yet distinctive and challenging childhood tumors. The uniqueness of this tumor depends on its biological markers, which classify neuroblastomas into favorable and unfavorable, with 5-year survival rates ranging from almost 100–30%. In this review, we focus on some biological factors that play major roles in neuroblastoma: MYCN, Trk, and ALK. The MYCN and Trk family genes have been studied for decades and are known to be crucial for the tumorigenesis and progression of neuroblastoma. ALK gene mutations have been recognized recently to be responsible for familial neuroblastomas. Each factor plays an important role in normal neural development, regulating cell proliferation or differentiation by activating several signaling pathways, and interacting with each other. These factors have been studied not only as prognostic factors, but also as targets of neuroblastoma therapy, and some clinical trials are ongoing. We review the basic aspects of MYCN, Trk, and ALK in both neural development and in neuroblastoma.

Keywords Neuroblastoma · MYCN · Trk · ALK

Introduction

Neuroblastoma is one of the most frequent solid tumors in children, occurring clinically in about 1 in 10,000 births, without racial predilection. Neuroblastoma develops in the sympathetic neurons and adrenal medulla, originating from the developmental sympathoadrenal linage derived from the neural crest. Neuroblastoma is known for its unique characteristics. Children under 1 year of age without any prognostic risk markers usually have favorable outcomes and even spontaneous regression of the tumor. However, tumors with certain prognostic markers are among the most challenging and refractory childhood malignancies. Thus, the 5-year survival rate ranges from almost 100% for favorable cases to 30% for progressive tumors with certain biological markers [1].

Embryonic tumors in childhood, such as neuroblastoma, have different characteristics from adult cancers because several developmental regulators of normal tissues contribute to the development of these tumors, which originate from developing cells in the associated organs. The tumorigenesis and progression of neuroblastoma are suspected to be closely related to disruption of the developmental mechanisms of sympathetic neurons and the adrenal medulla. Fewer genetic anomalies than those of adult cancers and perhaps more epigenetic dysfunction of gene regulation contribute to the tumorigenesis [2–4]. However, these genomic changes generally result in aggressive tumor progression. The MYCN and Trk families are the most well-studied genes in neuroblastoma research. These genes have been studied for decades in relation to their tumorigenesis or malignant potential. Anaplastic lymphoma kinase (ALK) has been recognized relatively recently to be related to familial neuroblastomas as well as some sporadic neuroblastomas [5, 6], suggesting new therapeutic options targeting this protein [7, 8]. These genes also play critical roles in the development of the nervous system and interact with each other in the developmental processes of organs. Aberrations in these genes can cause the retention of immaturity and the proliferative ability in neuroblast cells, disrupting their normal development. This review focuses on the roles of these genes in neural development as well as in neuroblastoma.

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MYCN

The MYCN gene was first reported in 1983–1984 as an oncogene located in the amplified region of genomic DNA in neuroblastoma as a gene related to the oncogenes *c-myc* and *v-myc* [9, 10]. MYCN is an MYC family gene, the expression of which is limited in neural systems [11]. The correlation between MYCN gene amplification and the prognosis of patients with neuroblastoma is well recognized [12]. This gene is now regarded as the most crucial prognostic maker of neuroblastoma.

MYCN amplification is found in 20% of all patients with neuroblastoma [13]. This genetic anomaly is found as a form of double minutes (DMs) or heterogeneously staining regions (HSRs) in neuroblastomas [9, 14], and the amplicon size ranges from 100 kb to more than 1 Mb [15]. The MYCN gene is located on the core 100–200 kb domain of the amplicon [15, 16]. Some co-amplified genes in this region, such as the DDX1, NAG or ALK genes, have been studied for their potential as oncogenes that contribute to neuroblastoma progression [17, 18], and mutations in the ALK gene have been found to be responsible for familial neuroblastoma [19, 20]. However, several studies have confirmed that MYCN is the most crucial gene and clearly related to the progression of sporadic neuroblastomas [15, 16]. The amplicons usually range from around 50- to 100-fold, although they can be several 100-folds in copy numbers. High levels of MYCN protein are the consequence of MYCN gene amplification [16], although MYCN expression can be high even without gene amplification [21], and it does not always correspond to the patient's prognosis [22]. However, high MYCN expression is still related to the immaturity or aggressive proliferation of cells. MYCN expression is well known to be suppressed in differentiating neuroblastoma cells by TrkA activation with its ligand NGF. In our recent study, we found that MYCN expression was suppressed by MEK inhibitors, which block the Ras–ERK pathway in some neuroblastoma cell lines [23]. We speculate that MYCN expression is partly regulated by the downstream of Ras–ERK.

The MYCN gene is located on chromosome 2p24 and encodes a 64-kDa protein. MYCN is a transcription factor with a basic helix-loop-helix motif, which forms a complex with the helix-loop-helix leucine zipper protein, Max, an MYC-associated factor, and binds to E-boxes around the target genes (Fig. 1a) [24]. MYCN can also repress gene expression independent of the E-box. Zinc finger protein Miz-1 recruits MYCN and Dnmt3a to DNA, forming a complex on the promoter of the target gene and causing gene silencing (Fig. 1b) [25–27]. Thereby, MYCN directly regulates the expression of target genes that maintain the pluripotency of cells and contributes to

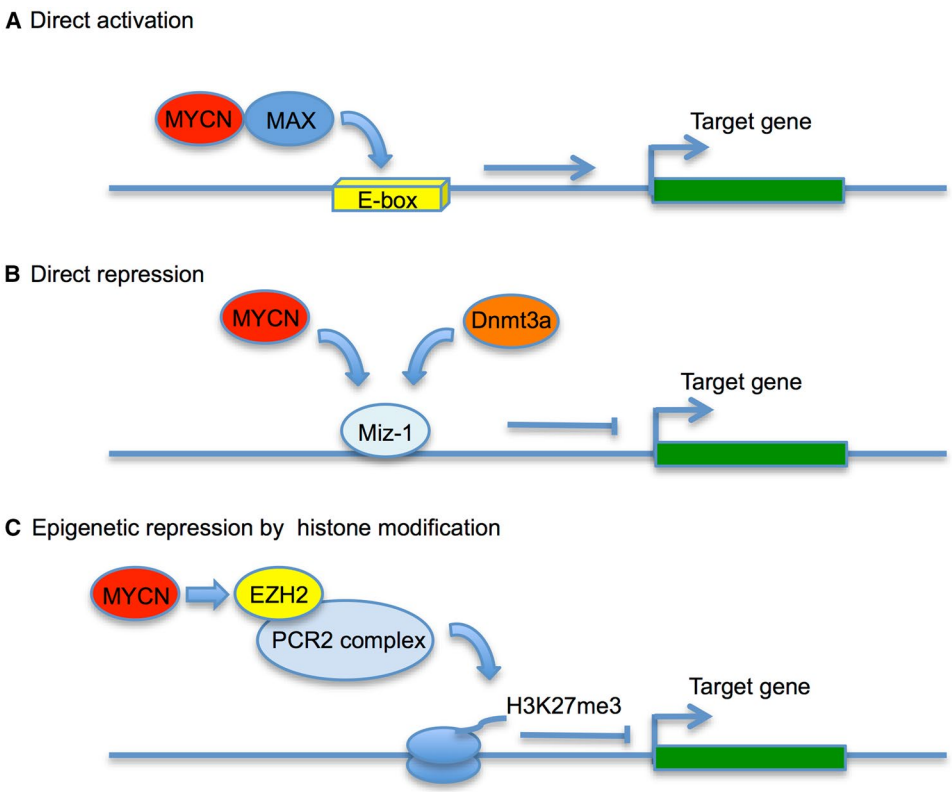

Fig. 1 Regulation of gene expression by MYCN. **a** Direct activation, **b** direct repression, and **c** epigenetic repression by histone modification

the cell proliferation or cell cycle progression. MYCN also interacts with DNA methyltransferase EZH2, which forms a PRC2 complex and regulates histone H3k27 methylation (Fig. 1c) [28, 29]. This chromosomal modification is crucial for the regulation of gene expression in developing tissues, implying that MYCN is also an epigenetic regulator of gene expression. The complementary expression of c-MYC and MYCN has also been reported [30], and both c-MYC and MYCN are key factors for maintaining the pluripotency of tumor cells or iPS cells [31].

MYCN expression is essential for normal neural development; however, while its expression is found in the process of neural crest cell development, it disappears in differentiated adult neural tissue. Wakamatsu et al. [11] analyzed MYCN expression in embryonal rats and detected expression in the dorsal root ganglia and sympathetic ganglia in the course of the migration and differentiation of neural crest cells. The MYCN expression is regulated downstream of the sonic hedgehog signaling [32] and IGF/PI3K pathways [33, 34], and the function of MYCN is also enhanced with the existence of a *Ras* mutation by being stabilized and/or translated downstream of Ras activation [35], which corresponds to our findings of MYCN suppression with MEK inhibitor in neuroblastoma cell lines [23].

The most well-established neuroblastoma mouse model is that of the MYCN-transgenic mouse. This mouse model is designed to express MYCN under the tyrosine hydroxylase promoter, which is upregulated in the sympathoadrenal system from the embryonal period [36]. Almost 100% of homogeneous mice and about 30% of heterogeneous mice harbor neuroblastoma-like tumors in sympathetic nodules [37]. This model is regarded as evidence that MYCN overexpression is crucial for the development of tumors in the sympathetic nervous system. Targeting MYCN in neuroblastoma treatment has been a major area of interest for researchers, but little progress has been made. The MYCN function can be inhibited using JQ1, a BET bromodomain protein inhibitor [38]. JQ1 inhibits the BET bromodomain protein, BRD4, which regulates the *MYC* family gene expression. MYCN expression is suppressed by JQ1 treatment in neuroblastoma cells. The clinical use of JQ1 is difficult because of its short half-life; however, similar BET inhibitors have shown potential utility in MYC target therapy [39].

In summary, MYCN amplification is one of the most crucial prognostic factors in neuroblastoma. As a transcriptional factor, MYCN regulates target gene expressions to maintain the pluripotency or proliferation of cells. Although MYCN is crucial for neuroblastoma tumorigenesis or proliferation, successful MYCN target therapy is still being developed.

Trks

Members of the Trk family—TrkA (NTRK1), TrkB (NTRK2), and TrkC (NTRK3)—are receptor tyrosine kinases (RTKs), the ligands of which are the neurotrophin family of growth factors (nerve growth factor [NGF], brain-derived neurotrophic factor [BDNF], neurotrophin-3 [NT-3] and neurotrophin-4/5 [NT-4/5]). Each receptor has an affinity preference for its ligand: NGF binds mainly to TrkA, BDNF or NT-4/5 binds to TrkB, and NT-3 binds to TrkC, although all neurotrophins have a weak affinity for p75, a TNF receptor superfamily [40, 41]. Although all neurotrophin-RTKs activate relatively common signaling cascades, the activation of each receptor results in a different outcome. NGF/TrkA is crucial for the differentiation of sympathetic and sensory neurons, and BDNF/TrkB is a key to the enhancement of the activity-stimulated excitation of neurons that is needed for memory development and maintenance [42–44]. The Neurotrophins-Trks reaction activates the downstream cascades of Ras/MAPK and PI3K/Akt. Both signaling pathways are crucial for cell survival and cell proliferation (Fig. 2).

The high expression of TrkA has been found to be a favorable marker in neuroblastomas showing differentiation or spontaneous regression, whereas the high expression of TrkB is a marker of unfavorable neuroblastoma and aggressive progression. BDNF/TrkB signaling stimulates cell survival and angiogenesis [45, 46]. However, interesting contradictions have been identified in these receptors. Some splicing variants exist in these receptor proteins, and shortly spliced TrkB protein is preferentially found in differentiating neuroblastomas [47, 48]. In contrast, a splicing variant of TrkA, to which NGF does not ligate, is found dominantly in unfavorable neuroblastomas [49]. TrkC is also expressed highly in favorable neuroblastomas or medulloblastomas, although the prognostic impact is less than that of TrkA or TrkB [50, 51]. p75 is reported to play a role in regulating the neurotrophin-receptor specificities, such as NGF to TrkA or BDNF to TrkB [52].

The Trk family members also play different roles in inducing neural cell apoptosis. For example, the existence of TrkA or TrkC without their ligands, but not TrkB, causes cell death [53]. Infantile neuroblastomas often show spontaneous regression or natural tumor differentiation to ganglioneuromas, and several reports have suggested that this phenomenon is induced by the existence of TrkA without its ligand NGF [54, 55]. One of the most well-studied Trk inhibitors is CEP-701 (Lestaurtinib). This compound is a multi-kinase inhibitor, which is known to block TrkB in the treatment of neuroblastoma. A Phase 1 study of this compound has been performed for refractory neuroblastomas [56, 57]. Several other Trk inhibitors are also being developed [58].

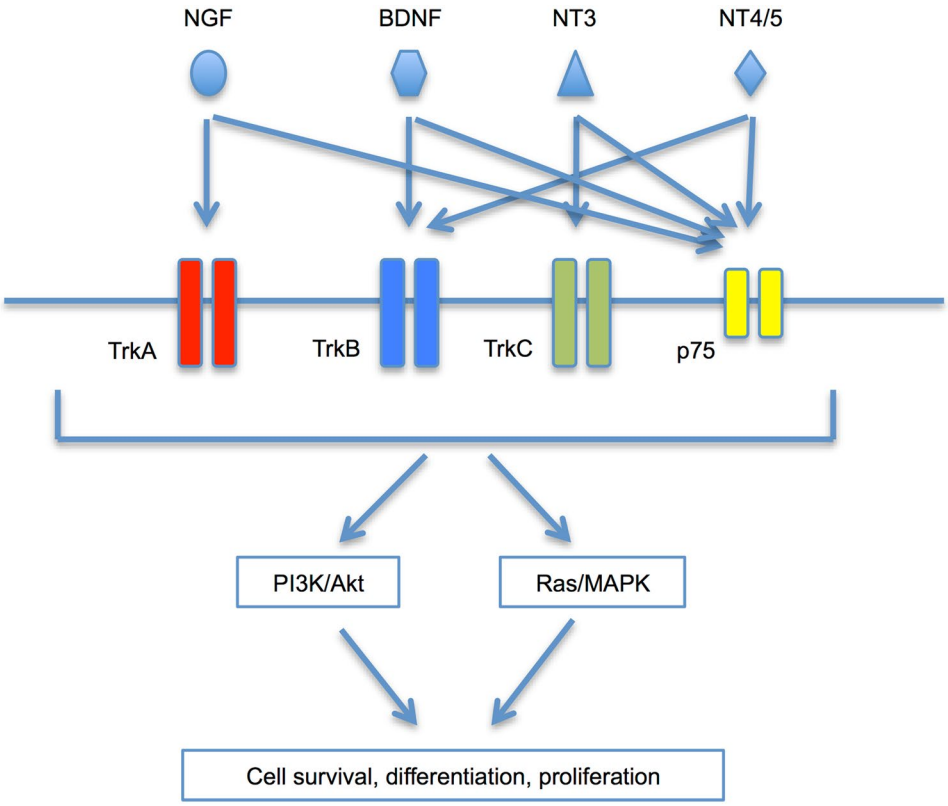

Fig. 2 Trk family receptors and ligands

In summary, the *Trk* family genes are crucial for neural development, and each plays a different role. They contribute to the cell proliferation or differentiation, as well as apoptosis in the nervous system or in tumors. The Trk signaling pathways may be the mechanism underlying spontaneous regression or targets of neuroblastoma therapy.

ALK

ALK is another receptor tyrosine kinase, with gene mutations found in most familial neuroblastomas as well as in 7–10% of sporadic neuroblastomas [6]. There are two hotspots of driving mutations for ALK found in neuroblastoma patients: ALK F1174 and ALK R1275 [59, 60]. Both mutations induce ALK auto-phosphorylation and continuous activation, which lead to the high tumorigenic potential of cells. Neural crest progenitor cells with overexpression of ALK F1174 or ALK R1275 showed a highly aggressive nature in vitro and in vivo [60]. The gene amplification is also found in several neuroblastoma cell lines that are sensitive to treatment with ALK inhibitors [19]. In contrast, the fusion proteins NPM–ALK, EML4–ALK and others are found in adult cancers, such as lung cancer [7, 61].

ALK is generally expressed in the central and peripheral nervous systems during embryonic development, although its normal function has not yet been fully clarified. ALK is a dependence receptor, the ligand of which is still unclear. Similar to TrkA or RET, ALK is also reported to lead cells to one of two opposite fates depending on the existence of its ligands. With its ligands, cells expressing ALK are anti-apoptotic and remain alive during differentiation or tumorigenesis, but without its ligands, ALK pushes cells toward apoptosis [62].

MYCN and RET are reportedly regulated by ALK. Umapathy et al. showed that ALK regulates the MYCN expression through the ERK5 pathway, one of the MAPK pathways. Mutated ALK promotes stabilization of the MYCN protein [63]. RET is also reported to be positively regulated by mutated ALK [64, 65], suggesting that RET may be a therapeutic target for the treatment of ALK-mutated neuroblastomas [65].

Clinical trials are being conducted to investigate several small-molecule ALK inhibitors such as crizotinib, which has antitumor effects on cancers with ALK mutations. One Phase 1 clinical trial on crizotinib in pediatric cancer patients finished recently [66]. However, cancer treatment with crizotinib can be made difficult by acquired resistance, so strategies to improve outcomes are being explored. The remarkable effects of next-generation ALK inhibitors against neuroblastomas have been shown both in vivo and in vitro [67].

In summary, ALK is a critical factor for neuroblastoma tumorigenesis and a potential target for therapy, depending on its mutation status.

Discussion

Neuroblastomas vary widely in their biology and clinical appearance. The three genes we discussed in this article have the most influence on the progression of the tumor. MYCN amplification has a strong prognostic impact and its existence is included among the factors for neuroblastoma risk classification. The Trk family expression has been studied in neuroblastoma research and is suspected to play a role in the spontaneous regression of infantile neuroblastomas. The involvement of ALK in the development of neuroblastomas has been recognized more recently than that of the other two genes. Since it has been discovered to play a role as an oncogene in adult cancers, ALK has been a target of anticancer treatment and inhibitors against it are being developed.

The biological findings that influence outcomes have been clarified; however, in reality, few contribute directly to improving the prognosis. The idea of targeting these prognostic factors is still of interest to neuroblastoma researchers. MYCN, Trks, and ALK are critical prognostic factors in neuroblastoma and these factors' activities crosslink with each other. Interactions between pathway activities make molecular-targeted therapy complicated. Studies have revealed many associated factors and working mechanisms in tumors. As such, novel strategies targeting specific pathways are required to improve the effectiveness of treatments.

Compliance with ethical standards

Conflict of interest Mayumi Higashi, Kohei Sakai, Shigeaki Fumino, Shigeyoshi Aoi, Taizo Furukawa and Tatsuro Tajiri have no conflicts of interest to declare.

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