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More than the genes, the tumor microenvironment in neuroblastoma

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Abstract

Neuroblastoma is the second most common solid tumor in children. Since the seminal discovery of the role of amplification of the MYCN oncogene in the pathogenesis of neuroblastoma in the 1980s, much focus has been on the contribution of genetic alterations in the progression of this cancer. However it is now clear that not only genetic events play a role but that the tumor microenvironment (TME) substantially contributes to the biology of neuroblastoma. In this article, we present a comprehensive review of the literature on the contribution of the TME to the ten hallmarks of cancer in neuroblastoma and discuss the mechanisms of communication between neuroblastoma cells and the TME that underlie the influence of the TME on neuroblastoma progression. We end our review by discussing how the knowledge acquired over the last two decades in this field is now leading to new clinical trials targeting the TME.

Keywords

Neuroblastoma; Tumor microenvironment; Hallmarks of cancer; Cell–cell communication

Introduction: neuroblastoma, a cancer not solely driven by genetic events

Neuroblastoma is the second most common solid tumor in children and accounts for 8–10% of childhood cancers in the USA and Europe. This cancer derives from neuroepithelial cells that migrate from the neural crest to form the sympathetic nervous system [1]. Children affected with this disease typically present with a posterior, paraspinal mass in the adrenal gland or along the sympathetic chain in the chest or abdomen. In approximately 50% of

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Conflict of interest

None.

children the disease presents as a locoregional tumor lacking amplification of the MYCN oncogene, and carries an excellent prognosis (>90% overall survival). However, the other half is considered clinically to be high-risk, either having tumors harboring MYCN amplification or being older (diagnosed in children older than 18 months of age) with metastatic disease irrespective of MYCN amplification. The children with high-risk disease have less than 50% chance of event-free long term survival despite an intensive therapy that combines myeloablative chemotherapy, radiation therapy, progenitor cell transplantation, surgery, isotretinoin and antibody-based immunotherapy [2,3]. The discovery in the early 1980s of a correlation between the amplification of the MYCN oncogene and advanced stage neuroblastoma [4–6] led to the anticipation of other genetic events. About 1–2% of patients with neuroblastoma have a family history of the disease and 2 specific genes, ALK and PHOX2B, have been shown to be mutated (gain of function in the case of ALK, loss of function in the case of PHOX2B) in 80% of the familial cases [7,8]. Genome-wide association studies further identified several gene polymorphisms associated with a low but significant risk of neuroblastoma, including BARD1, LMO1 and LIN28B [9]. However a recent genomic analysis of more than 200 neuroblastoma tumors revealed an unexpected low level of recurrent-driver mutations in neuroblastoma. These were mainly activation mutation and amplification of ALK (8% of the cases), activation mutations in PTPN11 (a tyrosine phosphatase), inactivating mutations in chromatin remodeling genes (ATRX and ARID1A) and activating mutations in NRAS in addition to amplification and activation mutations of MYCN [10–12]. A recent analysis in a subgroup of patients with MYCN non-amplified neuroblastoma revealed that metastatic neuroblastomas had higher infiltration of tumor-associated macrophages (TAM) than locoregional tumors. This analysis also revealed that metastatic tumors diagnosed in patients at age ≥ 18 months had higher expression of inflammation-related genes than those in patients diagnosed at age < 18 months. Expression of genes representing TAMs (CD33/CD16/IL-10/FCGR3) in addition to IL-6 receptor (IL-6R) contributed to 25% of the accuracy of a novel 14-gene tumor classification score [13]. Infiltration with Th2-driven macrophages expressing CD163 and CD206 was also recently observed in a subset of high-risk neuroblastoma tumors with deletion of chromosome 11q and high levels of prostaglandin-synthase and elevated levels of PGE2 [14].

In this article we will review the multiple mechanisms by which the TME regulates tumor progression and metastasis in neuroblastoma, focusing on the contribution of innate (TAM, neutrophils, NK, dendritic cells) and adaptive (T, B, and NKT) immune cells, tumor-associated fibroblasts (TAF), bone marrow-derived mesenchymal stromal cells (MSC), endothelial cells, Schwann cells, and the extracellular matrix (ECM). We will discuss various mechanisms of communication between neuroblastoma cells and the TME used by neuroblastoma cells to “educate” the TME and by TME cells to activate in neuroblastoma signaling pathways affecting their behavior. This review will conclude with a brief discussion on ongoing clinical trials in neuroblastoma patients that target the TME.

Role of the TME in the hallmarks of neuroblastoma

In their article of 2011, Weinberg and Hanahan identified eight critical biological processes controlling cancer progression that they designated “Hallmarks” [15]. These include the

ability (1) to sustain proliferative signals, (2) to evade growth-suppressors, (3) to invade and metastasize, (4) to enable replicative immortality, (5) to induce angiogenesis, (6) to resist cell death, (7) to escape immune destruction, and (8) to deregulate cellular metabolism. Genomic instability and tumor promoting inflammation were also recognized as two enabling characteristics. The contribution of the TME to these ten fundamental characteristics of cancer is now evident [16]. It is reviewed in the case of neuroblastoma in this section (Fig. 1).

Ability to sustain proliferative signals

The growth of neuroblastoma cells is regulated by several growth factors that interact with specific receptor tyrosine kinases and non-receptor tyrosine kinases present at their surface. Among the most extensively studied are the neurotrophin receptors – TrkA/NTRK1, TrkB/NTRK2 and TrkC/NTRK3 – that bind to a family of cognate ligands including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT3), respectively [17]. These receptors have different effects on proliferation, and whereas the TrkA receptor limits proliferation and promotes differentiation, the TrkB receptor drives proliferation. Activation of these receptors can occur either by an autocrine mechanism by growth factors produced by neuroblastoma cells, by a paracrine mechanism or by autoactivation [18]. Much has been learned on how the TME could participate in the NGFs/NGFR pathway in controlling neuroblastoma cell proliferation from studies on spontaneous regression and differentiation of neuroblastoma tumors [19]. Many neuroblastoma tumors that spontaneously regress or differentiate like ganglioneuroblastoma have a stroma rich in Schwann cells, and although the origin of these cells remains controversial (normal cells that infiltrate the tumor vs. malignant cells sharing the same genetic abnormalities as tumor cells), these cells are an abundant source of NGF and other neurotrophins and cause immature MYCN-amplified (A) and non-amplified (NA) neuroblastoma cell lines to differentiate into benign ganglioneuroblastoma and ganglioneuroma [20–22].

Another cytokine receptor whose function in neuroblastoma has been more recently elucidated is the receptor for interleukin-6 (IL-6R). High expression of this receptor in high-risk MYCN-NA human tumors was reported to be associated with poorer clinical outcomes [13]. Most human neuroblastoma cell lines (MYCN-A and NA) express both the gp130 and gp80 proteins that constitute the IL-6R [23]. They do not express the ligand IL-6 or the soluble agonistic IL-6 receptor (sIL-6R). However, MSC and TAM are an abundant source of IL-6 and sIL-6R in the TME and cooperate to activate signal transduction and activator of transcription (STAT)3 which promotes proliferation and survival in neuroblastoma cell lines [24].

The ECM also plays a role in controlling the growth of neuroblastoma cells. In most epithelial cancer an increased stiffness in the ECM (desmoplastic response) is associated with a stimulatory effect on cell proliferation [25], however it enhances neurogenesis, suppresses cell proliferation and reduces expression of MYCN in an MYCN-A cell line (SK-N-DZ), an effect that is enhanced by retinoic acid [26].

Ability to evade growth-suppressors

The major tumor suppressor pathway in neuroblastoma is p53. However, a striking feature is the low frequency (<2%) of *TP53* mutations at diagnosis and even at recurrence [27]. In contrast, alteration of the MDM2 (a p53 inhibitor that promotes p53 degradation) pathway seems to be the mechanism allowing escape from tumor suppression [28]. Evidence that the TME can dysregulate this pathway is currently missing although there is evidence that IL-6 can upregulate MDM2 in transformed and non-transformed cells [29] but this has not been examined in neuroblastoma.

Ability to enable replicative immortality

Like most cancers, neuroblastoma tumors express high levels of telomerase, and such high levels are typically associated with MYCN amplification and poor outcome [30]. Although considered for long to be an intrinsic property of tumor cells, there has been recent evidence demonstrating that telomerase activity in human neuroblastoma cell lines could be controlled by the TME and in particular by inflammatory monocytes/macrophages. It has been shown that exosome-mediated transfer of miR-21 from neuroblastoma cells to monocytes induces the release of miR-155 containing exosomes captured by neuroblastoma cells. Once in neuroblastoma cells, miR-155 directly targets TERF1, an inhibitor of telomerase whose silencing increases telomerase activity promoting chemoresistance in xenograft tumors [31].

Ability to invade and metastasize

The bone marrow, bone and liver are the most common sites of distant metastasis in patients with stage 4 neuroblastoma [32]. Some of the mechanisms involved in bone marrow and bone metastasis have been unraveled. Most human neuroblastoma cell lines derived from patients with high-risk disease (MYCN-A and NA) express the CXCR4 and CXCR7 receptors for the chemokine CXCL12, also known as stromal-derived factor-1 (SDF-1) [33,34]. Expression of CXCR4 in primary tumors also correlates with bone and bone marrow metastasis [35]. CXCR4 expression is associated with highly aggressive undifferentiated tumors, while CXCR7 expression is present in more differentiated and mature tumors. Whereas CXCR4 overexpression in neuroblastoma cells favors dissemination to the liver and the lungs, CXCR7 strongly promotes homing to the adrenal gland and the liver, and co-expression of CXCR4 and CXCR7 receptors significantly and selectively increases neuroblastoma dissemination toward the bone marrow [36,37]. MSC and osteoblasts are a major source of CXCL12/SDF-1 and thus contribute to a microenvironment that promotes bone marrow and bone metastasis [38].

Bone metastasis in neuroblastoma is associated with a predominant osteolytic process led by activation of osteoclasts [39]. Here also the TME plays a critical role. In bone metastasis, secretion of IL-6 by MSC triggered by neuroblastoma cell lines is a major factor promoting osteoclast activation and bone degradation [40]. The production of insulin-like growth factors (IGF-) 1 and -2 by neuroblastoma cell lines and their release from the bone contribute to this process. IGF acts on type I growth factor receptor IGF-1R expressed by preosteoclasts and promotes their activation [41]. High expression of IGF-1R in neuroblastoma cell lines increases adherence and homing to bone and bone metastasis [42].

Preosteoclasts express the receptor activator of NF κ B (RANK) which when interacting with RANK ligand (RANKL) produced by osteoblasts becomes activated and promotes osteoclast maturation and activity [43]. The mechanism of liver metastasis in neuroblastoma is much less understood although gastrin-releasing peptide receptor (GRP-R), expressed in metastatic neuroblastoma cells, may play a role. This receptor activates focal adhesion kinase (FAK), a critical downstream regulator of GRP-R that promotes liver metastasis. FAK expression correlates with GRP-R expression in tumors and cell lines (MYCN-A and NA) [44].

Ability to promote tumor vascularization

Both angiogenesis and vasculogenesis contribute to the vascularization of neuroblastoma tumors. As in most cancers, angiogenesis in neuroblastoma occurs through the production by tumor cells of several angiogenic factors such as vascular endothelial cell growth factor (VEGF), platelet-derived growth factor (PDGF) and fibro-blast growth factor (FGF) whose expression correlates with MYCN amplification and other markers of aggressiveness [45,46]. MYC, being a master regulator of vascular remodeling, has an important paracrine function in angiogenesis [47,48]. MYC knock out in mice is embryonically lethal as a consequence of defective hematopoiesis and vasculogenesis and endogenous suppression of MYC in a pancreatic islet tumor model in mice causes tumor regression through vascular collapse [49]. In neuroblastoma, MYCN upregulates the expression of VEGF [50] and driving MYCN degradation with inhibitors of PI3K/mTor inhibits tumor progression in TH-MYC transgenic mice, not only through MYCN downregulation but also through a paracrine blockade of angiogenesis [51]. There is also evidence that ALK controls angiogenesis via the production of VEGF as ALK knock-down in human neuroblastoma cell lines transduced with ALK mutant is associated with a marked reduction of VEGF secretion and blood vessel density in xenotransplanted tumors [52]. Although tumor cells are the main source of VEGF, murine and human neuroblastoma cell lines also stimulate the production of VEGF by MSC which promotes osteoblastogenesis through an intracrine mechanism [53]. Schwann cells, which are abundantly present in more differentiated stroma rich tumors, exert an anti-angiogenic function through the production of several inhibitors of angiogenesis such as tissue inhibitor of metalloproteinase 2 (TIMP-2), pigment-derived epithelial factor (PDEF) and secreted protein acidic rich in cysteine (SPARC) [54–56]. Whereas the presence of Schwann cells in tumors is typically associated with decreased vascularization, the presence of TAF (also inversely related to the presence of Schwann cells) is associated with increased vascularity, thus suggesting that these 2 cell types control the balance of angiogenesis in neuroblastoma [57].

VEGFR2 expressing endothelial progenitor cells (EPC) are recruited to tumors from the bone marrow by a mechanism that is dependent on the production of matrix metalloproteinase-9 (MMP-9) by myeloid cells [58]. MMP-9 promotes the release of soluble c-kit ligand that is necessary for the transition of EPC from a quiescent to a proliferative state in the bone marrow. MMP-9 promotes the recruitment of pericytes into the vasculature [59,60].

Ability to resist cell death

Loss of caspase-8 expression by gene silencing or gene deletion is a common feature of aggressive and metastatic neuroblastoma cell lines and patient tumors which allows to avoid programmed cell death [61]. However, when present, caspase-8 has a second function that is regulated by the TME. Paradoxically caspase-8 can promote migration and metastasis in neuroblastoma cell lines (NB 5, 7 and 16) in a manner that is not dependent on its proteolytic and apoptotic activity but that is regulated by contact with the ECM. Upon integrin ligation to ECM proteins, caspase-8 forms a complex with FAK, calpain2/CPN2 and calpastatin which, by disrupting CPN2/calpastatin interaction, activates CPN2. Activation of CPN2 enhances the cleavage of focal adhesion substrates and migration of MYCN-A and MYCN-NA human neuroblastoma cells lines *in vitro* [62]. There is also evidence that the TME promotes survival in neuroblastoma cell lines by upregulating anti-apoptotic proteins. For example, activation of STAT3 by IL-6 and sIL-6R produced by MSC and TAM induces the expression of several pro-survival proteins like survivin, MCL-1 and Bcl-XL that cause resistance to chemotherapeutic agents [24]. In contrast, IL-12 produced by Th1-driven TAM increases apoptosis in orthotopic murine neuroblastoma tumor models via tumor necrosis (TNF)- α and interferon (IFN)- γ which increase the expression of pro-apoptotic proteins, inhibit Akt phosphorylation and activate Bid [63].

Ability to escape the immune system

Neuroblastoma cells have developed multiple mechanisms to escape the immune system. Cell lines typically have low levels of expression of peptide presenting HLA class I molecules, which impairs target peptide recognition by cytotoxic T cells (CTL) [64–66]. The expression of major histocompatibility complex (MHC) I in neuroblastoma cell lines is controlled by the TME. For example, INF- γ produced by NK, NKT and T cells increases the expression of MHC I [65,67] and activation of NTRK1/TrkA upon ligation with NGF leads to an increased immunogenicity by overexpression of MHC I proteins [68], whereas MYCN amplification causes a downregulation of MHC I [69]. Neuroblastoma cells also escape immune attack by CTL and NK cells by producing soluble MHC I related chain (sMICA) and by expressing CD276/B7-H3, a co-stimulatory protein, which inhibit activation of NK cells and T cells via interaction with their receptors. Downregulation of the NKG2D ligands observed in human primary tumors and cell lines also contributes to immune evasion [70,71]. Neuroblastoma cell lines (6 out of 12) express low levels of HLA-G but induce monocytes to release soluble HLA-G, a protein that induces immune tolerance during pregnancy and is a ligand for the NK cell inhibitory receptor KIR2DL4 [72]. TAM further contribute to this immunosuppressive environment because they are a source of secreted immunosuppressive cytokines including IL-6, IL-10, and transforming growth factor (TGF) β 1. Despite this suppressive environment, NK cells, which in contrast to CTL do not depend on the expression of MHC I proteins on tumor cells, may be more effective although they still can be inhibited. Type 1 NKT cells do not depend on CD1d expression in neuroblastoma cells to be active in tumors [73]. When activated by IL-2, NK cells secrete IFN- γ and become cytotoxic via perforin-dependent and independent mechanisms [74]. Activated NKT cells secrete IL-2 which in turn activates NK cells [73] and eliminates TAM [75].

There is evidence that MYCN has a paracrine regulatory role on immune cells in the TME. For example, MYCN downregulates CCL2/MCP-1 (macrophage chemoattractant protein) which attracts NKT cells in tumors [76,77]. The contribution of immune check points such as PD-1/PD-L1 and CTLA-4/B7-1 to immunosuppression has just begun to be explored. In murine neuroblastoma models mAb targeting 4-1BB, CD40 and CTLA-4 induces tumor regression [78]. There is also pre-clinical evidence that PDL-1 blockade potentiates immunogenicity of human neuroblastoma cell lines [79].

Ability to deregulate cellular metabolism

Many cancers preferentially meet their energy requirements through the glycolytic pathway rather than via the more efficient oxidative phosphorylation pathway (the Warburg effect). Human neuroblastoma cell lines (MYCN-A and NA) *in vitro* preferentially use the glycolytic pathway in a way that is not dependent on MYCN [80]. The contribution of the TME to this effect has not been well explored and is thus poorly understood. There is some suggestion that dietary restriction leading toward low glucose concentration may inhibit the Warburg effect and increase cytotoxicity in neuroblastoma cell lines [81].

Genomic instability

Genomic instability has been extensively studied in neuroblastoma as discussed in our introduction. There is however little evidence so far that genomic instability can be affected by the TME. Considering that telomere-dependent chromosomal instability is highly prevalent in aggressive MYCN-NA neuroblastoma [82], it is conceivable that it could be affected by regulators of telomerase such as TERF1, whose expression in neuroblastoma cell lines has been shown to be dependent on an interactive loop between neuroblastoma cells and TAM [31].

Induction of a pro-tumorigenic inflammation

Innate immune cells such as monocytes/macrophages and neutrophils can create in neuroblastoma tumors an inflammatory environment that affects tumor progression and metastasis. There is evidence that neuroblastoma cells educate TAM toward a Th2-driven phenotype that supports their progression. The presence of macrophages in neuroblastoma tumors is an indicator of poor outcome and more aggressive disease in MYCN-NA high risk tumors [13,14], and blocking macrophage stimulatory factor (CSF-1) in CSF-1 negative xenotransplanted neuroblastoma tumors extends survival in tumor-bearing mice [83]. The presence of these macrophages is also associated with elevated levels of PGE2 and the presence of cancer-associated fibroblasts [14]. In contrast, neutrophils can be cytotoxic and inhibit the growth of human neuroblastoma cell lines (GD2 positive and negative) when in the presence of a chimeric anti-GD2 antibody but promote tumor cell growth in the absence of such antibody or if GD2 is not expressed [84,85].

There is thus growing evidence that the TME plays a regulatory role in neuroblastoma progression. The ultimate effect however is the result of dynamic and complex interactions between neuroblastoma cells and TME cells, leading toward an environment that is pro- or anti-tumorigenic. In the next section, we will review several mechanisms of communication

between neuroblastoma cells and TME cells and the ECM that regulate these interactions (Fig. 2).

Mechanisms of communication between neuroblastoma cells and TME cells

The reciprocal communication between neuroblastoma cells and the TME is based on 2 fundamental types of mechanism, (i) adhesion-dependent mechanisms that involve a contact between tumor cells and TME cells (cell–cell contact) or between tumor cells and components of the ECM (cell–ECM contact); and (ii) adhesion-independent mechanisms through the production of soluble products.

Adhesion-dependent mechanisms

Cell–TME contact—Neuroblastoma cell lines (SK-N-SH and LAN-1) are able to interact directly with endothelial cells [86] and leukocytes [87] as they express cell adhesion molecules (CAM) of the IgG superfamily like VCAM-1 and ICAM-1 which promote cell migration and metastasis. The expression of VCAM-1 and ICAM-1 in neuroblastoma cell lines (SK-N-SH and SK-N-MC) is upregulated by inflammatory cytokines, such as TNF- α and IFN- γ [88].

Neuroblastoma cells express CD56/NCAM which mediates their interaction with CD56/NCAM positive NK cells. It has been suggested that such interaction augments tumor cell lysis by NK cells but the importance of such function is not entirely clear [89–91].

Cell–ECM contact—Neuroblastoma cells interact with the ECM through the expression of several integrins. The effect that integrins have on neuroblastoma progression depends on the type of integrin expressed and the type of ECM protein involved. For example, the integrin $\alpha 5 \beta 1$ that mediates adhesion to collagen and fibronectin has an inhibitory function on migration. *In vitro* substrate adherent (S-type) neuroblastoma cells with high levels of $\alpha 5 \beta 1$ are less migratory and tumorigenic, whereas neuroblastic (N-type) neuroblastoma cells with low levels of integrin $\alpha 5 \beta 1$ do not attach to fibronectin and collagen IV, and are more migratory and tumorigenic. Consistently, blocking of $\alpha 5 \beta 1$ integrin in S-type cells promotes migration and the over expression of $\alpha 5 \beta 1$ integrin into N-type cells increases their attachment to fibronectin and collagen IV and inhibits migration [92,93]. IGF-1R stimulation decreases $\beta 1$ -integrin promoting cell migration and invasion [94]. There is also a reverse correlation between $\beta 1$ integrin and MYCN expression and progression in neuroblastoma cell lines and xenografts. Over-expression of MYCN in transfected SK-N-SH cells downregulates the expression of $\beta 1$ integrin [95,96]. Consistently, human neuroblastoma tumors with good prognosis have a high expression of $\alpha 5 \beta 1$ integrin, whereas in tumors that originate from patients with metastatic disease there is a lack of $\alpha 5 \beta 1$ integrin expression and high level of $\alpha v \beta 3$ integrin [97].

Other integrins promote migration. This is the case of $\alpha 3 \beta 1$ and $\alpha 1 \beta 1$ integrins that interact with laminin promoting migration but also neurite outgrowth in the N-type SH-SY5Y human neuroblastoma cell line in a manner that is dependent on O-mannosyl-linked

glycosylation events [98,99]. Binding of integrins to ECM proteins activates FAK and Src [100]. FAK-Src activation stimulates neuroblastoma cell motility but also recruits several adapter proteins such as talin and paxillin as well as the protease CPN2/calpain [101]. As previously discussed, caspase-8 is also recruited in this complex, is inactivated by Src-dependent phosphorylation of Tyr380 and activates CPN2, which promotes cell migration in apoptosis resistant cells [62,102,103]. Binding of integrins to fibronectin in mouse neuroblastoma cell lines also promotes the activation of cyclin E and cell proliferation [104]. $\alpha 4$ integrin is more frequently expressed in MYCN-NA human neuroblastoma cell lines and is selectively associated with a poorer outcome in MYCN-NA tumors. It interacts with an alternatively spliced variant of fibronectin, as well as with VCAM-1 promoting migration *in vitro* and metastasis *in vivo* [105].

CD44, a transmembrane glycoprotein that mediates cell adhesion to hyaluronan, is expressed by neuroblastoma cells. Its expression correlates with a non-metastatic phenotype and is commonly seen in MYCN-NA neuroblastoma tumors [106,107]. MYCN-A neuroblastoma cell lines (SHEP and ACN) do not express CD44 or express a non-functional variant of CD44 that does not bind to hyaluronan [108].

Disialoganglioside (GD2), a surface glycolipid present on neurons, peripheral nerve fibers, and skin melanocytes, is highly expressed in primary neuroblastoma tumors and is a target for immunotherapy [109]. GD2 facilitates the attachment of tumor cells to ECM proteins such as collagen, vitronectin, laminin, and fibronectin [110,111], which is blocked by the anti-GD2 mAb 14G2a leading to cell death [112].

Adhesion-independent mechanisms

The reciprocal communication between neuroblastoma cells and TME cells also involves soluble products providing paracrine and distant mechanisms of communication. Whereas much of the focus has been on the contribution of proteins there has been recent evidence that extracellular vesicles (EV) carrying not only proteins but also DNA and RNA could play an important role [31]. In this section, we will focus on some of the major chemokines, cytokines and growth factors specifically involved in paracrine interaction between neuroblastoma cells and stromal cells, illustrating how they are used by TME cells to activate specific signaling pathways in tumor cells and how they are used by tumor cells to “educate” the TME.

Axes of communication from TME cells to tumor cells

CXCL12/CXCR4/Akt axis: As previously discussed, the chemokine CXCL12/SDF-1, produced by MSC and osteoblasts in the bone marrow niche, plays an important role in the establishment of distant metastasis by attracting neuroblastoma cells that express CXCR4 and CXCR7 [38]. Interaction between CXCL12/SDF-1 with its receptor CXCR4 induces cell migration, proliferation and survival in an Akt-dependent mechanism that is inhibited by dipeptidyl peptidase IV, a cell surface serine protease present in some neuroblastoma cell lines [113].

IL-6/IL-6R/STAT3 axis: Neuroblastoma cell lines do not make IL-6, but upregulate its expression by MSC and TAM. In addition to activating osteoclasts and promoting osteolytic bone metastasis [40], IL-6 binding to its receptor IL-6R expressed by neuroblastoma cell lines exerts a pro-tumorigenic effect on tumor cells, enhancing growth, survival and drug resistance in a STAT3-dependent mechanism [23,24].

VEGF/VEGFR/Akt and ERK1/2 axis: Among the growth factors involved in the interaction between neuroblastoma cells and stromal cells is VEGF. Neuroblastoma cell lines, in particular MYCN-A, produce large amounts of VEGF but also stimulate its production in TME cells. Several studies reported the expression of VEGF receptors, primarily neuropilin-1 and 2, in neuroblastoma cell lines and some expression of VEGFR1 and VEGFR2 mRNA in state III tumors [114–117]. VEGF promotes cell survival of neuroblastoma cells via the PI3K/Akt pathway [118] and resistance to etoposide by activating ERK1/2 and upregulating BCL-2 [119].

IGF-1/IGFR/ERK1/2 and PI3K/Akt axis: IGF-1 is secreted by many cells including MSC [120] and endothelial cells [121]. It is also released from the bone matrix during osteolysis [122]. N-type neuroblastoma cell lines express the IGF-1 receptor (IGF-1R) [42]. Binding of IGF-1 to its receptor activates ERK1/2 and PI3K/Akt and promotes cell migration [94,123–125] and homing in the bone marrow [42]. It also stimulates neuroblastoma cell survival in SHEP cells and protects them from apoptosis through the upregulation of BCL-2 and inhibition of caspase-3 [126]. This effect could be particularly significant in MYCN-A neuroblastoma cell lines since there is an increased expression of IGF-1R in MYCN-transfected cells [127].

TGF- β /TGFBRI/SMAD axis: TGF- β is secreted by many TME cells such as MSC and TAM, and is also released in the bone environment upon bone degradation [128–131]. In addition to being a potent inhibitor of the immune system, TGF- β directly acts on neuroblastoma cells by affecting proliferation and differentiation in a way that depends on the receptors expressed. Neuroblastoma cells express various levels of TGFBRI (I, II and III) with expression of TGFBRII and III being inversely correlated with poor outcome. Consistently, TGF- β inhibits cell proliferation in human neuroblastoma cell lines expressing type II and III receptors [131] and overexpression of TGFBRII suppresses tumorigenesis [132]. TGFBRIII promotes differentiation and suppresses neuroblastoma proliferation whereas TGFBRI only promotes differentiation [132]. Furthermore, evidence that PI3K/Akt negatively regulates SMAD2/4 signaling in neuroblastoma suggests that an imbalance between PI3K/Akt and TGF- β /SMAD signaling is a mechanism promoting tumorigenesis in neuroblastoma [133].

PDGF/PDGF-R and PI3K/Akt and ERK1/2 axis: PDGF is secreted by endothelial cells [134], preosteoclasts [135], and can interact with neuroblastoma cell lines that express PDGFR. PDGFR activation stimulates migration, invasion and proliferation in PI3K and ERK dependent mechanisms [136].

Soluble products secreted by neuroblastoma cells that “educate” TME cells

Much less is known of the soluble products released by neuroblastoma cells that interact and educate TME cells. The contribution of neuroblastoma-derived VEGF to the autocrine (neuroblastoma cells) and paracrine (endothelial cells, MSC) activation of signaling pathways promoting tumor cell growth and angiogenesis is well known and has been discussed previously. Neuroblastoma cells secrete MCP-1/CCL2 that interacts with the CCR2 receptor expressed by type I NKT cells which attracts them in the tumor. The expression of MCP-1 by neuroblastoma cells is downregulated by MYCN thus preventing the recruitment of type I NKT cells and contributing to immune escape [76].

Human neuroblastoma cell lines produce galectin-3 binding protein (Gal-3BP), a sialoglycoprotein also present in neuroblastoma tumors that interacts with galectin-3 present in TME cells such as TAM and MSC. Gal-3BP interaction with Gal-3 induces the transcriptional activation of IL-6 in a RAS/MEK/ERK-dependent mechanism [137,138].

There is also recent evidence that the secretion of extracellular vesicles including exosomes by tumor cells is a mechanism used to educate TME cells and, for example, to induce the establishment of a pre-metastatic niche [139]. These vesicles contain a large variety of proteins, lipids, metabolites and nucleic acids including regulatory miR that act as shuttles mediating the communication between tumor cells and stromal cells in both directions. The protein content of the cargo of exosomes from several neuroblastoma cells includes, in addition to the exosomal markers (CD63, CD9, CD81, Hsp70, Hsp 90, Alix), GD2 disialoganglioside and proteins involved in tumor progression such as CD147, a multifunctional protein involved in invasion and metastasis, and CD276/B7-H3, an immune checkpoint protein that protects neuroblastoma cells from attack by NK cells [140]. The profile of miR content of exosome-like particles from two MYCN-A neuroblastoma cell lines was also recently reported showing that the exosomal miRNAs are associated with a range of cellular and molecular functions related to cell growth, cancer progression and drug resistance [31,141].

Therapeutic consideration

With a better knowledge of the contribution of the TME to neuroblastoma progression and of its mechanisms, clinical trials testing agents targeting the TME have been initiated. These trials follow three main strategies including (1) Direct targeting of TME cells that contribute to a protumorigenic environment, (2) Targeting signaling pathways activated by the TME in tumor cells or in TME cells (or both), and (3) Immunotherapy. A complete discussion of these strategies is beyond the scope of this review article but a few illustrative examples of ongoing Phase I/II clinical trials are given in Table 1.

Strategies targeting TME cells

Targeting osteoclasts with bisphosphonates like zoledronic acid has been tested in patients with bone metastasis and the results of a Phase I trial completed by the New Advances in Neuroblastoma Therapy (NANT) consortium demonstrated the safety and efficacy of zoledronic acid [142]. This agent is now tested with cyclophosphamide in a Phase I clinical

trial by Baylor College of Medicine (NCT00206388) and in combination with Interleukin-2 in refractory neuroblastoma by University of Alabama at Birmingham (NCT01404702).

Targeting endothelial cells in neuroblastoma has also been tested and a Phase I trial with bevacizumab, cyclophosphamide and zoledronic acid in patients with recurrent or refractory high-risk neuroblastoma has been recently completed by the NANT consortium (NCT00885326).

Strategies targeting pathways activated by the TME

These strategies typically combine a small molecule inhibitor of a signaling pathway with standard therapy. For example, sorafenib, a broad spectrum kinase inhibitor with pre-clinical activity in neuroblastoma [143,144], is presently tested in Phase I in combination with cyclophosphamide and topotecan in patients with relapsed and refractory neuroblastoma by NANT (NCT02298348). ZD6474, an inhibitor of VEGFR and EGFR, is also tested in Phase I alone and in combination with retinoic acid in neuroblastoma by M.D. Anderson Cancer Center (NCT00533169). Lestaurtinib (CEP-701), an inhibitor of JAK-2, TrkA, TrkB and TrkC, is actually tested in Phase I in young patients with recurrent or refractory high-risk neuroblastoma by NANT (NCT00084422). R(+)-XK469, an inhibitor of topoisomerase II- β and MEK/MAPK signaling kinases, is used in treating patients with advanced neuroblastoma in Phase I by NCI (NCT00028522). Temsirolimus, a rapamycin analog inhibitor of the Akt pathway, is presently tested in combination with irinotecan and temozolomide in patients with relapsed pediatric solid tumors including neuroblastoma [145] by the NCI (NCT01767194). SF1126, a dual PI3K/Akt and mTOR inhibitor, is tested in patients with refractory/recurrent neuroblastoma (NCT02337309). In addition, a combination of Rapamycin, mTOR inhibitor, with dasatinib is currently tested in patients with relapsed or refractory high-risk Neuroblastoma by the University of Regensburg (NCT01467986).

Immunotherapies

Whereas the human-mouse chimeric 14:18 anti-GD2 monoclonal antibody is now part of the standard therapy of neuroblastoma [3], other anti-GD2 antibodies are tested. The monoclonal antibody 3F8 is currently tested in a Phase II trial in combination with sargramostim in patients with neuroblastoma (NCT00072358). In addition, another clinical trial sponsored by Memorial Sloan Kettering Cancer Center is testing the antibody 3F8 in combination with GM-CSF (NCT00450307) [146]. The same antibody 3F8 coated in T cells to promote cytotoxicity is also the subject of a Phase I trial. Activated T cells armed with a GD2 bispecific antibody are tested in Phase I in children and young adults with neuroblastoma at the Barbara Ann Karmanos Cancer Institute (NCT02173093). New strategies using immunomodulators like lenalidomide [147], vaccine therapy and engineered T cells and NK cells are planned, often in combination with anti-GD2 immunotherapy, by several groups [148–150]. This is an area of intensive investigation.

Conclusion

After much focus on genetic aspects of neuroblastoma biology in the late 1990s and early 2000s, it is now clear that neuroblastoma is truly a disease of the seed and the soil, and that the TME significantly contributes in a favorable or unfavorable way to its progression. The observations summarized in this article raised the interesting and unexplored question of the potential interaction between genetic driver events in neuroblastoma and their influence through paracrine mechanisms on the TME. In other words, do genetic alterations in the seeds have the ability to influence the soil in a way that is critical for their development and survival? We have reviewed some evidence, for example, that MYCN and ALK could exert a paracrine control on angiogenesis. Thus targeting MYCN will have a dual effect on the seed and the soil.

As we better appreciate the tremendous level of complexity in the interactions between neuroblastoma cells and the TME, the challenge will be to distinguish interactions that play a more dominant role affecting both tumor and TME cells from those that have a less important function, to identify effective agents for these pathways and to develop biomarkers to monitor their effects. The study of the TME in neuroblastoma will continue to be an important field of investigation that lead to new therapies.

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Abbreviations

TME	Tumor microenvironment
TAM	Tumor-associated macrophages
TAF	Tumor-associated fibroblasts
MSC	Mesenchymal stromal cells
BM	Bone marrow
ECM	Extracellular matrix

References

1. Cheung NK, Dyer MA. Neuroblastoma: developmental biology, cancer genomics and immunotherapy. *Nat Rev Cancer*. 2013; 13:397–411. [PubMed: 23702928]
2. Matthay KK, Villablanca JG, Seeger RC, Stram DO, Harris RE, Ramsay NK, et al. Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid. Children's Cancer Group. *N Engl J Med*. 1999; 341:1165–1173. [PubMed: 10519894]
3. Yu AL, Gilman AL, Ozkaynak MF, London WB, Kreissman SG, Chen HX, et al. Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. *N Engl J Med*. 2010; 363:1324–1334. [PubMed: 20879881]

4. Schwab M, Alitalo K, Klempnauer KH, Varmus HE, Bishop JM, Gilbert F, et al. Amplified DNA with limited homology to myc cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumour. *Nature*. 1983; 305:245–248.
5. Brodeur GM, Seeger RC, Schwab M, Varmus HE, Bishop JM. Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. *Science*. 1984; 224:1121–1124. [PubMed: 6719137]
6. Seeger RC, Brodeur GM, Sather H, Dalton A, Siegel SE, Wong KY, et al. Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. *N Engl J Med*. 1985; 313:1111–1116. [PubMed: 4047115]
7. Janoueix-Lerosey I, Lequin D, Brugieres L, Ribeiro A, de Pontual L, Combaret V, et al. Somatic and germline activating mutations of the ALK kinase receptor in neuroblastoma. *Nature*. 2008; 455:967–970. [PubMed: 18923523]
8. Trochet D, Bourdeaut F, Janoueix-Lerosey I, Deville A, de Pontual L, Schleiermacher G, et al. Germline mutations of the paired-like homeobox 2B (PHOX2B) gene in neuroblastoma. *Am J Hum Genet*. 2004; 74:761–764. [PubMed: 15024693]
9. Nguyen LB, Diskin SJ, Capasso M, Wang K, Diamond MA, Glessner J, et al. Phenotype restricted genome-wide association study using a gene-centric approach identifies three low-risk neuroblastoma susceptibility loci. *PloS Genet*. 2011; 7:e1002026. [PubMed: 21436895]
10. Pugh TJ, Morozova O, Attiyeh EF, Asgharzadeh S, Wei JS, Auclair D, et al. The genetic landscape of high-risk neuroblastoma. *Nat Genet*. 2013; 45:279–284. [PubMed: 23334666]
11. Cheung NK, Zhang J, Lu C, Parker M, Bahrami A, Tickoo SK, et al. Association of age at diagnosis and genetic mutations in patients with neuroblastoma. *JAMA*. 2012; 307:1062–1071. [PubMed: 22416102]
12. Valentijn LJ, Koster J, Haneveld F, Aissa RA, van Sluis P, Broekmans ME, et al. Functional MYCN signature predicts outcome of neuroblastoma irrespective of MYCN amplification. *Proc Natl Acad Sci USA*. 2012; 109:19190–19195. [PubMed: 23091029]
13. Asgharzadeh S, Salo JA, Ji L, Oberthuer A, Fischer M, Berthold F, et al. Clinical significance of tumor-associated inflammatory cells in metastatic neuroblastoma. *J Clin Oncol*. 2012; 30:3525–3532. [PubMed: 22927533]
14. Larsson K, Kock A, Idborg H, Arsenian Henriksson M, Martinsson T, Johnsen JJ, et al. COX/mPGES-1/PGE2 pathway depicts an inflammatory-dependent high-risk neuroblastoma subset. *Proc Natl Acad Sci USA*. 2015; 112:8070–8075. [PubMed: 26080408]
15. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011; 144:646–674. [PubMed: 21376230]
16. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell*. 2012; 21:309–322. [PubMed: 22439926]
17. Brodeur GM. Neuroblastoma: biological insights into a clinical enigma. *Nat Rev Cancer*. 2003; 3:203–216. [PubMed: 12612655]
18. Brodeur GM, Minturn JE, Ho R, Simpson AM, Iyer R, Varela CR, et al. Trk receptor expression and inhibition in neuroblastomas. *Clin Cancer Res*. 2009; 15:3244–3250. [PubMed: 19417027]
19. Brodeur GM, Bagatell R. Mechanisms of neuroblastoma regression. *Nat Rev Clin Oncol*. 2014; 11:704–713. [PubMed: 25331179]
20. Ambros IM, Zellner A, Roald B, Amann G, Ladenstein R, Printz D, et al. Role of ploidy, chromosome 1p, and Schwann cells in the maturation of neuroblastoma [see comments]. *N Engl J Med*. 1996; 334:1505–1511. [PubMed: 8618605]
21. Brodeur GM. Schwann cells as antineuroblastoma agents. *N Engl J Med*. 1996; 334:1537–1539. [PubMed: 8618612]
22. Kwiatkowski JL, Rutkowski JL, Yamashiro DJ, Tennekoon GI, Brodeur GM. Schwann cell-conditioned medium promotes neuroblastoma survival and differentiation. *Cancer Res*. 1998; 58:4602–4606. [PubMed: 9788610]
23. Ara T, Song L, Shimada H, Keshelava N, Russell HV, Metelitsa LS, et al. Interleukin-6 in the bone marrow microenvironment promotes the growth and survival of neuroblastoma cells. *Cancer Res*. 2009; 69:329–337. [PubMed: 19118018]

24. Ara T, Nakata R, Sheard MA, Shimada H, Buettner R, Groshen SG, et al. Critical role of STAT3 in IL-6-mediated drug resistance in human neuroblastoma. *Cancer Res.* 2013; 73:3852–3864. [PubMed: 23633489]
25. Weaver VM, Roskelley CD. Extracellular matrix: the central regulator of cell and tissue homeostasis. *Trends Cell Biol.* 1997; 7:40–42. [PubMed: 17708898]
26. Lam WA, Cao L, Umesh V, Keung AJ, Sen S, Kumar S. Extracellular matrix rigidity modulates neuroblastoma cell differentiation and N-myc expression. *Mol Cancer.* 2010; 9:35. [PubMed: 20144241]
27. Tweddle DA, Pearson AD, Haber M, Norris MD, Xue C, Flemming C, et al. The p53 pathway and its inactivation in neuroblastoma. *Cancer Lett.* 2003; 197:93–98. [PubMed: 12880966]
28. Van Maerken T, Vandesompele J, Rihani A, De Paepe A, Speleman F. Escape from p53-mediated tumor surveillance in neuroblastoma: switching off the p14(ARF)-MDM2-p53 axis. *Cell Death Differ.* 2009; 16:1563–1572. [PubMed: 19779493]
29. Brighenti E, Calabrese C, Liguori G, Giannone FA, Trere D, Montanaro L, et al. Interleukin 6 downregulates p53 expression and activity by stimulating ribosome biogenesis: a new pathway connecting inflammation to cancer. *Oncogene.* 2014; 33:4396–4406. [PubMed: 24531714]
30. Hiyama E, Hiyama K, Yokoyama T, Matsuura Y, Piatyszek MA, Shay JW. Correlating telomerase activity levels with human neuroblastoma outcomes. *Nat Med.* 1995; 1:249–255. [PubMed: 7585042]
31. Challagundla KB, Wise PM, Neviani P, Chava H, Murtadha M, Xu T, et al. Exosome-mediated transfer of microRNAs within the tumor microenvironment and neuroblastoma resistance to chemotherapy. *J Natl Cancer Inst.* 2015; 107
32. Dubois SG, Kalika Y, Lukens JN, Brodeur GM, Seeger RC, Atkinson JB, et al. Metastatic sites in stage IV and IVS neuroblastoma correlate with age, tumor biology, and survival. *J Pediatr Hematol Oncol.* 1999; 21:181–189. [PubMed: 10363850]
33. Carlisle AJ, Lyttle CA, Carlisle RY, Maris JM. CXCR4 expression heterogeneity in neuroblastoma cells due to ligand-independent regulation. *Mol Cancer.* 2009; 8:126. [PubMed: 20028517]
34. Liberman J, Sartelet H, Flahaut M, Muhlethaler-Mottet A, Coulon A, Nyalendo C, et al. Involvement of the CXCR7/CXCR4/CXCL12 axis in the malignant progression of human neuroblastoma. *PLoS ONE.* 2012; 7:e43665. [PubMed: 22916293]
35. Russell HV, Hicks J, Okcu MF, Nuchtern JG. CXCR4 expression in neuroblastoma primary tumors is associated with clinical presentation of bone and bone marrow metastases. *J Pediatr Surg.* 2004; 39:1506–1511. [PubMed: 15486895]
36. Muhlethaler-Mottet A, Liberman J, Ascencio K, Flahaut M, Balmas Bourlout K, Yan P, et al. The CXCR4/CXCR7/CXCL12 axis is involved in a secondary but complex control of neuroblastoma metastatic cell homing. *PLoS ONE.* 2015; 10:e0125616. [PubMed: 25955316]
37. Geminder H, Sagi-Assif O, Goldberg L, Meshel T, Rechavi G, Witz IP, et al. A possible role for CXCR4 and its ligand, the CXC chemokine stromal cell-derived factor-1, in the development of bone marrow metastases in neuroblastoma. *J Immunol.* 2001; 167:4747–4757. [PubMed: 11591806]
38. Ma M, Ye JY, Deng R, Dee CM, Chan GC. Mesenchymal stromal cells may enhance metastasis of neuroblastoma via SDF-1/CXCR4 and SDF-1/CXCR7 signaling. *Cancer Lett.* 2011; 312:1–10. [PubMed: 21906874]
39. Sohara Y, Shimada H, Scadeng M, Pollack H, Yamada S, Ye W, et al. Lytic bone lesions in human neuroblastoma xenograft involve osteoclast recruitment and are inhibited by bisphosphonate. *Cancer Res.* 2003; 63:3026–3031. [PubMed: 12810621]
40. Sohara Y, Shimada H, Minkin C, Erdreich-Epstein A, Nolte JA, DeClerck YA. Bone marrow mesenchymal stem cells provide an alternate pathway of osteoclast activation and bone destruction by cancer cells. *Cancer Res.* 2005; 65:1129–1135. [PubMed: 15734993]
41. Avnet S, Salerno M, Quacquarello G, Granchi D, Giunti A, Baldini N. IGF2 derived from SH-SY5Y neuroblastoma cells induces the osteoclastogenesis of human monocytic precursors. *Exp Cell Res.* 2011; 317:2147–2158. [PubMed: 21672538]

42. van Golen CM, Schwab TS, Kim B, Soules ME, Su OS, Fung K, et al. Insulin-like growth factor-I receptor expression regulates neuroblastoma metastasis to bone. *Cancer Res.* 2006; 66:6570–6578. [PubMed: 16818629]
43. Granchi D, Amato I, Battistelli L, Avnet S, Capaccioli S, Papucci L, et al. In vitro blockade of receptor activator of nuclear factor-kappaB ligand prevents osteoclastogenesis induced by neuroblastoma cells. *Int J Cancer.* 2004; 111:829–838. [PubMed: 15300794]
44. Lee S, Qiao J, Paul P, O'Connor KL, Evers MB, Chung DH. FAK is a critical regulator of neuroblastoma liver metastasis. *Oncotarget.* 2012; 3:1576–1587. [PubMed: 23211542]
45. Chlenski A, Liu S, Cohn SL. The regulation of angiogenesis in neuroblastoma. *Cancer Lett.* 2003; 197:47–52. [PubMed: 12880959]
46. Chesler L, Goldenberg DD, Seales IT, Satchi-Fainaro R, Grimmer M, Collins R, et al. Malignant progression and blockade of angiogenesis in a murine transgenic model of neuroblastoma. *Cancer Res.* 2007; 67:9435–9442. [PubMed: 17909053]
47. Baudino TA, McKay C, Pendeville-Samain H, Nilsson JA, Maclean KH, White EL, et al. c-Myc is essential for vasculogenesis and angiogenesis during development and tumor progression. *Genes Dev.* 2002; 16:2530–2543. [PubMed: 12368264]
48. Whitfield JR, Soucek L. Tumor microenvironment: becoming sick of Myc. *Cell Mol Life Sci.* 2012; 69:931–934. [PubMed: 22033838]
49. Sodik NM, Swigart LB, Karnezis AN, Hanahan D, Evan GI, Soucek L. Endogenous Myc maintains the tumor microenvironment. *Genes Dev.* 2011; 25:907–916. [PubMed: 21478273]
50. Kang J, Rychahou PG, Ishola TA, Mourot JM, Evers BM, Chung DH. N-myc is a novel regulator of PI3K-mediated VEGF expression in neuroblastoma. *Oncogene.* 2008; 27:3999–4007. [PubMed: 18278068]
51. Chantry YH, Gustafson WC, Itsara M, Persson A, Hackett CS, Grimmer M, et al. Paracrine signaling through MYCN enhances tumor-vascular interactions in neuroblastoma. *Sci Transl Med.* 2012; 4:115ra113.
52. Di Paolo D, Ambrogio C, Pastorino F, Brignole C, Martinengo C, Carosio R, et al. Selective therapeutic targeting of the anaplastic lymphoma kinase with liposomal siRNA induces apoptosis and inhibits angiogenesis in neuroblastoma. *Mol Ther.* 2011; 19:2201–2212. [PubMed: 21829174]
53. HaDuong JH, Blavier L, Baniwal SK, Frenkel B, Malvar J, Punj V, et al. Interaction between bone marrow stromal cells and neuroblastoma cells leads to a VEGFA-mediated osteoblastogenesis. *Int J Cancer.* 2015; 137:797–809. [PubMed: 25648303]
54. Chlenski A, Liu S, Crawford SE, Volpert OV, DeVries GH, Evangelista A, et al. SPARC is a key Schwannian-derived inhibitor controlling neuroblastoma tumor angiogenesis. *Cancer Res.* 2002; 62:7357–7363. [PubMed: 12499280]
55. Huang D, Rutkowski JL, Brodeur GM, Chou PM, Kwiatkowski JL, Babbo A, et al. Schwann cell-conditioned medium inhibits angiogenesis. *Cancer Res.* 2000; 60:5966–5971. [PubMed: 11085514]
56. Chlenski A, Liu S, Baker LJ, Yang Q, Tian Y, Salwen HR, et al. Neuroblastoma angiogenesis is inhibited with a folded synthetic molecule corresponding to the epidermal growth factor-like module of the follistatin domain of SPARC. *Cancer Res.* 2004; 64:7420–7425. [PubMed: 15492265]
57. Zeine R, Salwen HR, Peddinti R, Tian Y, Guerrero L, Yang Q, et al. Presence of cancer-associated fibroblasts inversely correlates with Schwannian stroma in neuroblastoma tumors. *Mod Pathol.* 2009; 22:950–958. [PubMed: 19407854]
58. Jodele S, Chantrain CF, Blavier L, Lutzko C, Crooks GM, Shimada H, et al. The contribution of bone marrow-derived cells to the tumor vasculature in neuroblastoma is matrix metalloproteinase-9 dependent. *Cancer Res.* 2005; 65:3200–3208. [PubMed: 15833851]
59. Chantrain CF, Shimada H, Jodele S, Groshen S, Ye W, Shalinsky DR, et al. Stromal matrix metalloproteinase-9 regulates the vascular architecture in neuroblastoma by promoting pericyte recruitment. *Cancer Res.* 2004; 64:1675–1686. [PubMed: 14996727]
60. Chantrain CF, Henriot P, Jodele S, Emonard H, Feron O, Courtoy PJ, et al. Mechanisms of pericyte recruitment in tumour angiogenesis: a new role for metalloproteinases. *Eur J Cancer.* 2006; 42:310–318. [PubMed: 16406506]

61. Teitz T, Lahti JM, Kidd VJ. Aggressive childhood neuroblastomas do not express caspase-8: an important component of programmed cell death. *J Mol Med.* 2001; 79:428–436. [PubMed: 11511973]
62. Barbero S, Mielgo A, Torres V, Teitz T, Shields DJ, Mikolon D, et al. Caspase-8 association with the focal adhesion complex promotes tumor cell migration and metastasis. *Cancer Res.* 2009; 69:3755–3763. [PubMed: 19383910]
63. Khan T, Hixon JA, Stauffer JK, Lincoln E, Back TC, Brenner J, et al. Therapeutic modulation of Akt activity and antitumor efficacy of interleukin-12 against orthotopic murine neuroblastoma. *J Natl Cancer Inst.* 2006; 98:190–202. [PubMed: 16449679]
64. Lampson LA, Fisher CA, Whelan JP. Striking paucity of HLA-A, B, C and beta 2-microglobulin on human neuroblastoma cell lines. *J Immunol.* 1983; 130:2471–2478. [PubMed: 6187860]
65. Lampson LA, Fisher CA. Weak HLA and beta 2-microglobulin expression of neuronal cell lines can be modulated by interferon. *Proc Natl Acad Sci USA.* 1984; 81:6476–6480. [PubMed: 6436814]
66. Main EK, Lampson LA, Hart MK, Kornbluth J, Wilson DB. Human neuroblastoma cell lines are susceptible to lysis by natural killer cells but not by cytotoxic T lymphocytes. *J Immunol.* 1985; 135:242–246. [PubMed: 3158702]
67. Handgretinger R, Kimmig A, Lang P, Daurer B, Kuci S, Bruchelt G, et al. Interferon-gamma upregulates the susceptibility of human neuroblastoma cells to interleukin-2-activated natural killer cells. *Nat Immun Cell Growth Regul.* 1989; 8:189–196. [PubMed: 2507905]
68. Pajtlér KW, Rebmann V, Lindemann M, Schulte JH, Schulte S, Stauder M, et al. Expression of NTRK1/TrkA affects immunogenicity of neuroblastoma cells. *Int J Cancer.* 2013; 133:908–919. [PubMed: 23400852]
69. Bernards R, Dessain SK, Weinberg RA. N-myc amplification causes down-modulation of MHC class I antigen expression in neuroblastoma. *Cell.* 1986; 47:667–674. [PubMed: 3096575]
70. Raffaghello L, Prigione I, Airolidi I, Camoriano M, Levreri I, Gambini C, et al. Downregulation and/or release of NKG2D ligands as immune evasion strategy of human neuroblastoma. *Neoplasia.* 2004; 6:558–568. [PubMed: 15548365]
71. Leitner J, Klauser C, Pickl WF, Stockl J, Majdic O, Bardet AF, et al. B7-H3 is a potent inhibitor of human T-cell activation: no evidence for B7-H3 and TREM2 interaction. *Eur J Immunol.* 2009; 39:1754–1764. [PubMed: 19544488]
72. Morandi F, Levreri I, Bocca P, Galleni B, Raffaghello L, Ferrone S, et al. Human neuroblastoma cells trigger an immunosuppressive program in monocytes by stimulating soluble HLA-G release. *Cancer Res.* 2007; 67:6433–6441. [PubMed: 17616704]
73. Metelitsa LS. Anti-tumor potential of type-I NKT cells against CD1d-positive and CD1d-negative tumors in humans. *Clin Immunol.* 2011; 140:119–129. [PubMed: 21095162]
74. Bottino C, Dondero A, Bellora F, Moretta L, Locatelli F, Pistoia V, et al. Natural killer cells and neuroblastoma: tumor recognition, escape mechanisms, and possible novel immunotherapeutic approaches. *Front Immunol.* 2014; 5:56. [PubMed: 24575100]
75. Song L, Asgharzadeh S, Salo J, Engell K, Wu HW, Spoto R, et al. Valpha24-invariant NKT cells mediate antitumor activity via killing of tumor-associated macrophages. *J Clin Invest.* 2009; 119:1524–1536. [PubMed: 19411762]
76. Metelitsa LS, Wu HW, Wang H, Yang Y, Warsi Z, Asgharzadeh S, et al. Natural killer T cells infiltrate neuroblastomas expressing the chemokine CCL2. *J Exp Med.* 2004; 199:1213–1221. [PubMed: 15123743]
77. Song L, Ara T, Wu HW, Woo CW, Reynolds CP, Seeger RC, et al. Oncogene MYCN regulates localization of NKT cells to the site of disease in neuroblastoma. *J Clin Invest.* 2007; 117:2702–2712. [PubMed: 17710228]
78. Williams EL, Dunn SN, James S, Johnson PW, Cragg MS, Glennie MJ, et al. Immunomodulatory monoclonal antibodies combined with peptide vaccination provide potent immunotherapy in an aggressive murine neuroblastoma model. *Clin Cancer Res.* 2013; 19:3545–3555. [PubMed: 23649004]
79. Boes M, Meyer-Wentrup F. TLR3 triggering regulates PD-L1 (CD274) expression in human neuroblastoma cells. *Cancer Lett.* 2015; 361:49–56. [PubMed: 25697485]

80. Smith DJ, Cossins LR, Hatzinisiriou I, Haber M, Nagley P. Lack of correlation between MYCN expression and the Warburg effect in neuroblastoma cell lines. *BMC Cancer*. 2008; 8:259. [PubMed: 18789162]
81. Navratilova J, Hankeova T, Benes P, Smarda J. Low-glucose conditions of tumor microenvironment enhance cytotoxicity of tetrathiomolybdate to neuroblastoma cells. *Nutr Cancer*. 2013; 65:702–710. [PubMed: 23859038]
82. Lundberg G, Sehic D, Lansberg JK, Ora I, Frigyesi A, Castel V, et al. Alternative lengthening of telomeres—an enhanced chromosomal instability in aggressive non-MYCN amplified and telomere elongated neuroblastomas. *Genes Chromosomes Cancer*. 2011; 50:250–262. [PubMed: 21319260]
83. Abraham D, Zins K, Sioud M, Lucas T, Schafer R, Stanley ER, et al. Stromal cell-derived CSF-1 blockade prolongs xenograft survival of CSF-1-negative neuroblastoma. *Int J Cancer*. 2010; 126:1339–1352. [PubMed: 19711348]
84. Chen RL, Reynolds CP, Seeger RC. Neutrophils are cytotoxic and growth-inhibiting for neuroblastoma cells with an anti-GD2 antibody but, without cytotoxicity, can be growth-stimulating. *Cancer Immunol Immunother*. 2000; 48:603–612. [PubMed: 10663607]
85. Michon J, Moutel S, Barbet J, Romet-Lemonne JL, Deo YM, Fridman WH, et al. In vitro killing of neuroblastoma cells by neutrophils derived from granulocyte colony-stimulating factor-treated cancer patients using an anti-disialoganglioside/anti-Fc gamma RI bispecific antibody. *Blood*. 1995; 86:1124–1130. [PubMed: 7542496]
86. Schwankhaus N, Gathmann C, Wicklein D, Riecken K, Schumacher U, Valentiner U. Cell adhesion molecules in metastatic neuroblastoma models. *Clin Exp Metastasis*. 2014; 31:483–496. [PubMed: 24549749]
87. Birdsall HH, Lane C, Ramser MN, Anderson DC. Induction of VCAM-1 and ICAM-1 on human neural cells and mechanisms of mononuclear leukocyte adherence. *J Immunol*. 1992; 148:2717–2723. [PubMed: 1374096]
88. Chuluyan HE, Lang BJ, Yoshimura T, Kenney JS, Issekutz AC. Chemokine production and adhesion molecule expression by neural cells exposed to IL-1, TNF alpha and interferon gamma. *Life Sci*. 1998; 63:1939–1952. [PubMed: 9825772]
89. Winter C, Pawel B, Seiser E, Zhao H, Raabe E, Wang Q, et al. Neural cell adhesion molecule (NCAM) isoform expression is associated with neuroblastoma differentiation status. *Pediatr Blood Cancer*. 2008; 51:10–16. [PubMed: 18213713]
90. Phimister E, Kiely F, Kemshead JT, Patel K. Expression of neural cell adhesion molecule (NCAM) isoforms in neuroblastoma. *J Clin Pathol*. 1991; 44:580–585. [PubMed: 1856291]
91. Lanier LL, Chang C, Azuma M, Ruitenberg JJ, Hemperly JJ, Phillips JH. Molecular and functional analysis of human natural killer cell-associated neural cell adhesion molecule (N-CAM/CD56). *J Immunol*. 1991; 146:4421–4426. [PubMed: 1710251]
92. Ciccarone V, Spengler BA, Meyers MB, Biedler JL, Ross RA. Phenotypic diversification in human neuroblastoma cells: expression of distinct neural crest lineages. *Cancer Res*. 1989; 49:219–225. [PubMed: 2535691]
93. Yoshihara T, Esumi N, Humphries MJ, Imashuku S. Unique expression of integrin fibronectin receptors in human neuroblastoma cell lines. *Int J Cancer*. 1992; 51:620–626. [PubMed: 1534785]
94. Meyer A, van Golen CM, Kim B, van Golen KL, Feldman EL. Integrin expression regulates neuroblastoma attachment and migration. *Neoplasia*. 2004; 6:332–342. [PubMed: 15256055]
95. Judware R, Culp LA. Over-expression of transfected N-myc oncogene in human SKNSH neuroblastoma cells down-regulates expression of beta 1 integrin subunit. *Oncogene*. 1995; 11:2599–2607. [PubMed: 8545117]
96. Judware R, Lechner R, Culp LA. Inverse expressions of the N-myc oncogene and beta 1 integrin in human neuroblastoma: relationships to disease progression in a nude mouse model system. *Clin Exp Metastasis*. 1995; 13:123–133. [PubMed: 7533687]
97. Favrot MC, Combaret V, Goillot E, Lutz P, Frappaz D, Thiesse P, et al. Expression of integrin receptors on 45 clinical neuroblastoma specimens. *Int J Cancer*. 1991; 49:347–355. [PubMed: 1917132]

98. Smith BE, Bradshaw AD, Choi ES, Rouselle P, Wayner EA, Clegg DO. Human SY5Y neuroblastoma cell interactions with laminin isoforms: neurite outgrowth on laminin-5 is mediated by integrin alpha 3 beta 1. *Cell Adhes Commun.* 1996; 3:451–462. [PubMed: 8807189]
99. Abbott KL, Troupe K, Lee I, Pierce M. Integrin-dependent neuroblastoma cell adhesion and migration on laminin is regulated by expression levels of two enzymes in the O-mannosyl-linked glycosylation pathway, PomGnT1 and GnT-Vb. *Exp Cell Res.* 2006; 312:2837–2850. [PubMed: 16857188]
100. Mitra SK, Hanson DA, Schlaepfer DD. Focal adhesion kinase: in command and control of cell motility. *Nat Rev Mol Cell Biol.* 2005; 6:56–68. [PubMed: 15688067]
101. Wu L, Bernard-Trifilo JA, Lim Y, Lim ST, Mitra SK, Uryu S, et al. Distinct FAK-Src activation events promote alpha5beta1 and alpha4beta1 integrin-stimulated neuroblastoma cell motility. *Oncogene.* 2008; 27:1439–1448. [PubMed: 17828307]
102. Cursi S, Rufini A, Stagni V, Condo I, Matafora V, Bachi A, et al. Src kinase phosphorylates Caspase-8 on Tyr380: a novel mechanism of apoptosis suppression. *EMBO J.* 2006; 25:1895–1905. [PubMed: 16619028]
103. Helfer B, Boswell BC, Finlay D, Cipres A, Vuori K, Bong Kang T, et al. Caspase-8 promotes cell motility and calpain activity under nonapoptotic conditions. *Cancer Res.* 2006; 66:4273–4278. [PubMed: 16618751]
104. Hulleman E, Bijvelt JJ, Verkleij AJ, Verrips CT, Boonstra J. Integrin signaling at the M/G1 transition induces expression of cyclin E. *Exp Cell Res.* 1999; 253:422–431. [PubMed: 10585265]
105. Young SA, McCabe KE, Bartakova A, Delaney J, Pizzo DP, Newbury RO, et al. Integrin alpha4 enhances metastasis and may be associated with poor prognosis in MYCN low neuroblastoma. *PLoS ONE.* 2015; 10:e0120815. [PubMed: 25973900]
106. Combaret V, Gross N, Lasset C, Frappaz D, Beretta-Brognara C, Philip T, et al. Clinical relevance of CD44 cell surface expression and MYCN gene amplification in neuroblastoma. *Eur J Cancer.* 1997; 33:2101–2105. [PubMed: 9516862]
107. Kramer K, Cheung NK, Gerald WL, LaQuaglia M, Kushner BH, LeClerc JM, et al. Correlation of MYCN amplification, Trk-A and CD44 expression with clinical stage in 250 patients with neuroblastoma. *Eur J Cancer.* 1997; 33:2098–2100. [PubMed: 9516861]
108. Gross N, Balmas K, Brognara CB. Absence of functional CD44 hyaluronan receptor on human NMYC-amplified neuroblastoma cells. *Cancer Res.* 1997; 57:1387–1393. [PubMed: 9102228]
109. Wu ZL, Schwartz E, Seeger R, Ladisch S. Expression of GD2 ganglioside by untreated primary human neuroblastomas. *Cancer Res.* 1986; 46:440–443. [PubMed: 3940209]
110. Kazarian T, Jabbar AA, Wen FQ, Patel DA, Valentino LA. Gangliosides regulate tumor cell adhesion to collagen. *Clin Exp Metastasis.* 2003; 20:311–319. [PubMed: 12856718]
111. Cheresch DA, Pierschbacher MD, Herzig MA, Mujoo K. Disialogangliosides GD2 and GD3 are involved in the attachment of human melanoma and neuroblastoma cells to extracellular matrix proteins. *J Cell Biol.* 1986; 102:688–696. [PubMed: 3005335]
112. Kowalczyk A, Gil M, Horwacik I, Odrowaz Z, Kozbor D, Rokita H. The GD2-specific 14G2a monoclonal antibody induces apoptosis and enhances cytotoxicity of chemotherapeutic drugs in IMR-32 human neuroblastoma cells. *Cancer Lett.* 2009; 281:171–182. [PubMed: 19339105]
113. Arscott WT, LaBauve AE, May V, Wesley UV. Suppression of neuroblastoma growth by dipeptidyl peptidase IV: relevance of chemokine regulation and caspase activation. *Oncogene.* 2009; 28:479–491. [PubMed: 18978811]
114. Meister B, Grünebach F, Bautz F, Brugger W, Fink FM, Kanz L, et al. Expression of vascular endothelial growth factor (VEGF) and its receptors in human neuroblastoma. *Eur J Cancer.* 1999; 35:445–449. [PubMed: 10448297]
115. Beierle EA, Dai W, Langham MR Jr, Copeland EM 3rd, Chen MK. Expression of VEGF receptors in cocultured neuroblastoma cells. *J Surg Res.* 2004; 119:56–65. [PubMed: 15126083]
116. Fakhari M, Pullirsch D, Abraham D, Paya K, Hofbauer R, Holzfeind P, et al. Selective upregulation of vascular endothelial growth factor receptors neuropilin-1 and -2 in human neuroblastoma. *Cancer.* 2002; 94:258–263. [PubMed: 11815985]

117. Fakhari M, Pullirsch D, Paya K, Abraham D, Hofbauer R, Aharinejad S. Upregulation of vascular endothelial growth factor receptors is associated with advanced neuroblastoma. *J Pediatr Surg.* 2002; 37:582–587. [PubMed: 11912515]
118. Beierle EA, Nagaram A, Dai W, Iyengar M, Chen MK. VEGF-mediated survivin expression in neuroblastoma cells. *J Surg Res.* 2005; 127:21–28. [PubMed: 15882878]
119. Das B, Yeager H, Tsuchida R, Torkin R, Gee MF, Thorner PS, et al. A hypoxia-driven vascular endothelial growth factor/Flt1 autocrine loop interacts with hypoxia-inducible factor-1alpha through mitogen-activated protein kinase/extracellular signal-regulated kinase 1/2 pathway in neuroblastoma. *Cancer Res.* 2005; 65:7267–7275. [PubMed: 16103078]
120. Roodman GD. Role of stromal-derived cytokines and growth factors in bone metastasis. *Cancer.* 2003; 97:733–738. [PubMed: 12548570]
121. Wang J, Tang Y, Zhang W, Zhao H, Wang R, Yan Y, et al. Insulin-like growth factor-1 secreted by brain microvascular endothelial cells attenuates neuron injury upon ischemia. *FEBS J.* 2013; 280:3658–3668. [PubMed: 23721666]
122. Boyce BF, Yoneda T, Guise TA. Factors regulating the growth of metastatic cancer in bone. *Endocr Relat Cancer.* 1999; 6:333–347. [PubMed: 10516850]
123. Kim B, van Golen CM, Feldman EL. Insulin-like growth factor-I signaling in human neuroblastoma cells. *Oncogene.* 2004; 23:130–141. [PubMed: 14712218]
124. Puglianiello A, Germani D, Rossi P, Cianfarani S. IGF-I stimulates chemotaxis of human neuroblasts. Involvement of type I IGF receptor, IGF binding proteins, phosphatidylinositol-3 kinase pathway and plasmin system. *J Endocrinol.* 2000; 165:123–131. [PubMed: 10750042]
125. Meyer GE, Shelden E, Kim B, Feldman EL. Insulin-like growth factor I stimulates motility in human neuroblastoma cells. *Oncogene.* 2001; 20:7542–7550. [PubMed: 11709726]
126. van Golen CM, Castle VP, Feldman EL. IGF-I receptor activation and BCL-2 overexpression prevent early apoptotic events in human neuroblastoma. *Cell Death Differ.* 2000; 7:654–665. [PubMed: 10889510]
127. Chambery D, Mohseni-Zadeh S, De Galle B, Babajko S. N-myc regulation of type I insulin-like growth factor receptor in a human neuroblastoma cell line. *Cancer Res.* 1999; 59:2898–2902. [PubMed: 10383152]
128. Assoian RK, Fleurdelys BE, Stevenson HC, Miller PJ, Madtes DK, Raines EW, et al. Expression and secretion of type beta transforming growth factor by activated human macrophages. *Proc Natl Acad Sci USA.* 1987; 84:6020–6024. [PubMed: 2888109]
129. Wahl SM, McCartney-Francis N, Allen JB, Dougherty EB, Dougherty SF. Macrophage production of TGF-beta and regulation by TGF-beta. *Ann N Y Acad Sci.* 1990; 593:188–196. [PubMed: 1695824]
130. Crane JL, Cao X. Bone marrow mesenchymal stem cells and TGF-beta signaling in bone remodeling. *J Clin Invest.* 2014; 124:466–472. [PubMed: 24487640]
131. Scarpa S, Coppa A, Ragano-Caracciolo M, Mincione G, Giuffrida A, Modesti A, et al. Transforming growth factor beta regulates differentiation and proliferation of human neuroblastoma. *Exp Cell Res.* 1996; 229:147–154. [PubMed: 8940258]
132. Turco A, Scarpa S, Coppa A, Baccheschi G, Palumbo C, Leonetti C, et al. Increased TGFbeta type II receptor expression suppresses the malignant phenotype and induces differentiation of human neuroblastoma cells. *Exp Cell Res.* 2000; 255:77–85. [PubMed: 10666336]
133. Lynch J, Fay J, Meehan M, Bryan K, Watters KM, Murphy DM, et al. MiRNA-335 suppresses neuroblastoma cell invasiveness by direct targeting of multiple genes from the non-canonical TGF-beta signalling pathway. *Carcinogenesis.* 2012; 33:976–985. [PubMed: 22382496]
134. Abramsson A, Lindblom P, Betsholtz C. Endothelial and nonendothelial sources of PDGF-B regulate pericyte recruitment and influence vascular pattern formation in tumors. *J Clin Invest.* 2003; 112:1142–1151. [PubMed: 14561699]
135. Xie H, Cui Z, Wang L, Xia Z, Hu Y, Xian L, et al. PDGF-BB secreted by preosteoclasts induces angiogenesis during coupling with osteogenesis. *Nat Med.* 2014; 20:1270–1278. [PubMed: 25282358]

136. Matsui T, Sano K, Tsukamoto T, Ito M, Takaishi T, Nakata H, et al. Human neuroblastoma cells express alpha and beta platelet-derived growth factor receptors coupling with neurotrophic and chemotactic signaling. *J Clin Invest.* 1993; 92:1153–1160. [PubMed: 8376577]
137. Fukaya Y, Shimada H, Wang LC, Zandi E, DeClerck YA. Identification of Gal-3 binding protein as a factor secreted by tumor cells that stimulates interleukin-6 expression in the bone marrow stroma. *J Biol Chem.* 2008; 283:18573–18581. [PubMed: 18450743]
138. Silverman AM, Nakata R, Shimada H, Sposto R, DeClerck YA. A galectin-3-dependent pathway upregulates interleukin-6 in the microenvironment of human neuroblastoma. *Cancer Res.* 2012; 72:2228–2238. [PubMed: 22389450]
139. Peinado H, Aleckovic M, Lavotshkin S, Matei I, Costa-Silva B, Moreno-Bueno G, et al. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat Med.* 2012; 18:883–891. [PubMed: 22635005]
140. Marimpietri D, Petretto A, Raffaghello L, Pezzolo A, Gagliani C, Tacchetti C, et al. Proteome profiling of neuroblastoma-derived exosomes reveal the expression of proteins potentially involved in tumor progression. *PLoS ONE.* 2013; 8:e75054. [PubMed: 24069378]
141. Haug BH, Hald OH, Utne P, Roth SA, Lokke C, Flaegstad T, et al. Exosome-like extracellular vesicles from MYCN-amplified neuroblastoma cells contain oncogenic miRNAs. *Anticancer Res.* 2015; 35:2521–2530. [PubMed: 25964525]
142. Russell HV, Groshen SG, Ara T, DeClerck YA, Hawkins R, Jackson HA, et al. A phase I study of zoledronic acid and low-dose cyclophosphamide in recurrent/refractory neuroblastoma: a new approaches to neuroblastoma therapy (NANT) study. *Pediatr Blood Cancer.* 2011; 57:275–282. [PubMed: 21671363]
143. Chai H, Luo AZ, Weerasinghe P, Brown RE. Sorafenib downregulates ERK/Akt and STAT3 survival pathways and induces apoptosis in a human neuroblastoma cell line. *Int J Clin Exp Pathol.* 2010; 3:408–415. [PubMed: 20490331]
144. Yang F, Jove V, Buettner R, Xin H, Wu J, Wang Y, et al. Sorafenib inhibits endogenous and IL-6/S1P induced JAK2-STAT3 signaling in human neuroblastoma, associated with growth suppression and apoptosis. *Cancer Biol Ther.* 2012; 13:534–541. [PubMed: 22406995]
145. Geoerger B, Kieran MW, Grupp S, Perek D, Clancy J, Krygowski M, et al. Phase II trial of temsirolimus in children with high-grade glioma, neuroblastoma and rhabdomyosarcoma. *Eur J Cancer.* 2012; 48:253–262. [PubMed: 22033322]
146. Kushner BH, Kramer K, Modak S, Cheung NK. Successful multifold dose escalation of anti-GD2 monoclonal antibody 3F8 in patients with neuroblastoma: a phase I study. *J Clin Oncol.* 2011; 29:1168–1174. [PubMed: 21343563]
147. Xu Y, Sun J, Sheard MA, Tran HC, Wan Z, Liu WY, et al. Lenalidomide overcomes suppression of human natural killer cell anti-tumor functions by neuroblastoma microenvironment-associated IL-6 and TGFbeta1. *Cancer Immunol Immunother.* 2013; 62:1637–1648. [PubMed: 23982484]
148. Louis CU, Savoldo B, Dotti G, Pule M, Yvon E, Myers GD, et al. Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. *Blood.* 2011; 118:6050–6056. [PubMed: 21984804]
149. Liu Y, Wu HW, Sheard MA, Sposto R, Somanchi SS, Cooper LJ, et al. Growth and activation of natural killer cells ex vivo from children with neuroblastoma for adoptive cell therapy. *Clin Cancer Res.* 2013; 19:2132–2143. [PubMed: 23378384]
150. Esser R, Muller T, Stefes D, Kloess S, Seidel D, Gillies SD, et al. NK cells engineered to express a GD2-specific antigen receptor display built-in ADCC-like activity against tumour cells of neuroectodermal origin. *J Cell Mol Med.* 2012; 16:569–581. [PubMed: 21595822]

Fig. 1. Diagram summarizing the contribution of the cells and ECM in the TME to the ten hallmarks of cancer shown at the center of the wheel. The central graph was reproduced from Hanahan and Weinberg [15].

Fig. 2. Diagram summarizing the major mechanisms of communication between neuroblastoma cells and TME cells and the ECM and pathways they activate in neuroblastoma cells.

Table 1

Registered Phase I/II clinical trials in neuroblastoma that target the TME. The list was generated from data on clinicaltrials.gov.

Category	Pathway(s)	Molecular target(s)	Study	Phase	Sponsor	Number of clinical trial
Tumor microenvironment	Osteoclast survival	Farnesyl pyrophosphate synthase	Zoledronic Acid (ZOMETA) With Cyclophosphamide With Neuroblastoma and Cortical Bone Involvement	Phase I	Baylor College of Medicine	NCT00206388
	Osteoclast survival	Farnesyl pyrophosphate synthase	Pilot Study of Zoledronic Acid and Interleukin-2 for Refractory Pediatric Neuroblastoma	Phase I	University of Alabama at Birmingham	NCT01404702
	VEGF signaling and osteoclast survival	VEGF and farnesyl pyrophosphate synthase	N2007-02: Bevacizumab, Cyclophosphamide, & Zoledronic Acid in Patients With Refractory High-Risk Neuroblastoma	Phase I	New Approaches to Neuroblastoma Therapy Consortium	NCT00885326
	MAPK and STAT signaling	RAF/MEK/ERK and STAT-3	Sorafenib and Cyclophosphamide/Topotecan in Patients With Relapsed and Refractory Neuroblastoma (N2013-02)	Phase I	New Approaches to Neuroblastoma Therapy Consortium	NCT02298348
Pathways activated by the TME	VEGFR and EGFR signaling	VEGFR and EGFR signaling	ZD6474 Alone and in Combination With Retinoic Acid in Pediatric Neuroblastoma	Phase I	M.D. Anderson Cancer Center	NCT00533169
	JAK/STAT and FLT3 signaling	JAK-2, TrkA, TrkB, TrkC,	N2001-03: CEP-701 in Treating Young Patients With Recurrent or Refractory High-Risk Neuroblastoma	Phase I	New Approaches to Neuroblastoma Therapy Consortium	NCT00084422
	MEK/MAPK signaling	Topoisomerase II- β and MEK/MAPK	R(+)-XK469 in Treating Patients With Advanced Neuroblastoma	Phase I	National Cancer Institute	NCT00028522
	AKT signaling	mTOR	Irinotecan Hydrochloride and Temozolomide With Temsirolimus or Dinutuximab in Treating Younger Patients With Refractory or Relapsed Neuroblastoma	Phase II	National Cancer Institute	NCT01767194
Immunotherapy	AKT signaling	PI3K and mTOR	SF1126 for Patients With Relapsed for Refractory Neuroblastoma	Phase I	New Approaches to Neuroblastoma Therapy Consortium	NCT02337309
	AKT signaling and Src family tyrosine kinase	mTOR and BCR/ABL and Src tyrosine kinases	Multimodal Molecular Targeted Therapy to Treat Relapsed or Refractory High-risk Neuroblastoma	Phase II	University of Regensburg	NCT01467986
	GD2	GD2	Monoclonal Antibody 3F8 and Sargramostim in Treating Patients With Neuroblastoma	Phase II	Memorial Sloan Kettering Cancer Center	NCT00072358

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Category	Pathway(s)	Molecular target(s)	Study	Phase	Sponsor	Number of clinical trial
	GD2	GD2	Monoclonal Antibody 3F8 and GM-CSF in Treating Young Patients With High-Risk, Refractory or Relapsed Neuroblastoma	Phase I	Memorial Sloan Kettering Cancer Center	NCT00450307
	GD2	GD2	Activated T Cells Armed With GD2 Bispecific Antibody in Children and Young Adults With Neuroblastoma and Osteosarcoma	Phase II	Barbara Ann Karmanos Cancer Institute	NCT02173093