

Neuroblastoma and MYCN

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Neuroblastoma, the most common extracranial solid tumor of childhood, is thought to originate from undifferentiated neural crest cells. Amplification of the MYC family member, *MYCN*, is found in ~25% of cases and correlates with high-risk disease and poor prognosis. Currently, amplification of *MYCN* remains the best-characterized genetic marker of risk in neuroblastoma. This article reviews roles for *MYCN* in neuroblastoma and highlights recent identification of other driver mutations. Strategies to target *MYCN* at the level of protein stability and transcription are also reviewed.

Neuroblastoma, described by James Wright in 1910, was named because cells were associated with fibrils in arrangements similar to neuroblasts. Nine of 12 cases were in children, suggesting the disease manifests early in life from primitive undifferentiated cells (Wright 1910). Today, neuroblastoma ranks as the most common cancer in infants (<1 year old), with 90% of cases diagnosed by age 5. The primary tumor is frequently located in tissues originating from the sympathetic nervous system, adrenal medulla, or paraspinal ganglia, and metastases are found in a majority of cases at diagnosis, consistent with an origin from multipotent migratory neural crest cells.

high-risk patients have <50% event-free survival rate, and there is also a subset of “ultra-high” risk patients who do not respond to therapy (Maris et al. 2007; Matthay et al. 2012). Current treatment for high-risk patients includes intensive and toxic chemotherapy, followed by surgical resection, myeloablation and autologous stem cell rescue, radiation, and intensive biologic/immunotherapy. Although most high-risk patients initially respond to chemotherapy, the majority relapse and succumb to therapy-resistant disease. Established characteristics for high-risk neuroblastoma patients include age, unfavorable histopathology, loss of heterozygosity for chromosome 1p or 11q, and amplification of *MYCN* (Mueller and Matthay 2009).

RISK IN NEUROBLASTOMA

Risk in neuroblastoma is classified as low, intermediate, or high. Although low- and intermediate-risk patients generally have a favorable outcome (~80%–95% event-free survival rate),

MYCN VERSUS MYC

MYCN was identified in 1983 as an amplified gene homologous to *v-myc* but distinct from



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MYC in human neuroblastoma (Kohl et al. 1983; Schwab et al. 1983). Structurally, the coding regions of both *MYC* and *MYCN* are highly homologous (Fig. 1A), with long 5' and 3' untranslated regions (UTRs) and gene products at similar sizes (~50–55 kDa) (Kohl et al. 1986; Stanton et al. 1986). *MYC* and *MYCN* proteins both heterodimerize with *MAX* at consensus E-box sequences (CANNTG), and both proteins have conserved regions for DNA-protein and protein-protein interactions (reviewed in Meyer and Penn 2008). Although a role for *MYC* in *trans*-repression (through heterodimerization with *MIZ1*) is well established (see Wiese et al. 2013), comparatively less is known about *trans*-repression by *MYCN* (Akter et al. 2011; Iraci et al. 2011).

Biologically, *MYCN*, like *MYC*, was found to promote transformation in rat embryo fibroblasts and induced proliferation and cell cycle

progression in quiescent fibroblasts (Fig. 1B) (Schwab et al. 1985; Yancopoulos et al. 1985; Cavalieri and Goldfarb 1988). Mouse embryonic stem cells (ESCs) homozygous for deletion of either *MYC* or *MYCN* showed normal morphology, and did not show aberrant proliferation or differentiation compared to wild-type ESCs, presumably because *MYC* and *MYCN* can compensate for each other (Charron et al. 1992; Davis et al. 1993; Sawai et al. 1993). Further supporting this idea of redundancy is the ability of *MYCN* knocked-in at the *MYC* locus to rescue embryonic lethality and to restore immune functions in *MYC* knockout mice (although *MYCN* animals were smaller, developed dystrophy of skeletal muscles, and showed differences in growth responses in some cell types; Malynn et al. 2000). Taken together, these findings suggest that *MYC* and *MYCN* show prominent, albeit incomplete, redundancy.

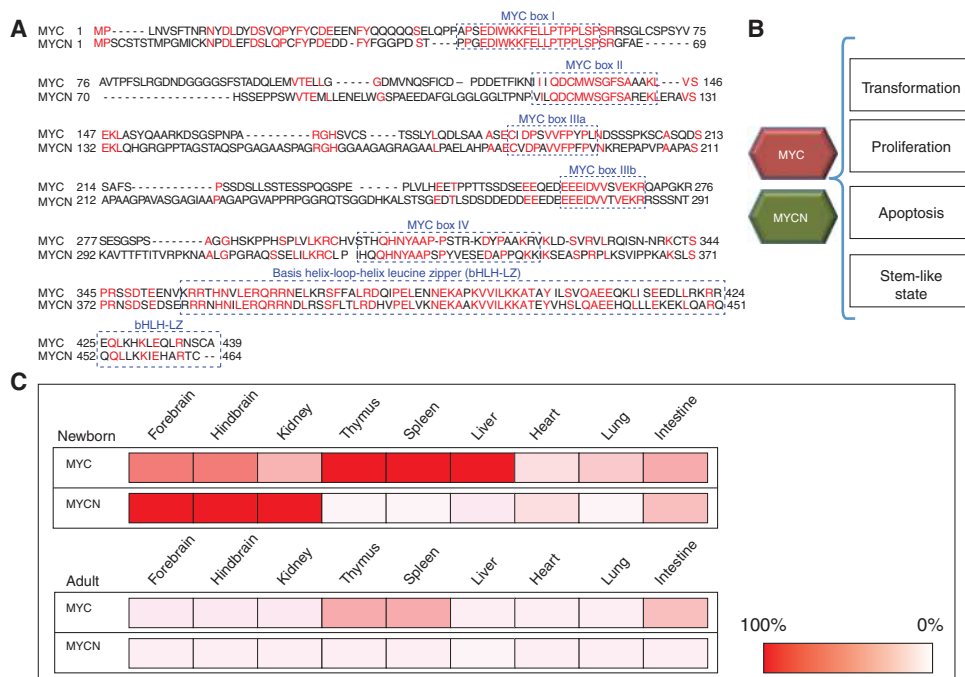


Figure 1. Similarities and differences between *MYC* and *MYCN*. *MYC* and *MYCN* are similar both in (A) structure (homologous sequences in red), and (B) biological functions. However, *MYC* and *MYCN* differ in (C) the spatiotemporal expression levels. In particular, *MYCN* is preferentially expressed in neural tissue, whereas *MYC* is more ubiquitously expressed. Expression of *MYC* and *MYCN* at each tissue is based on a relative percentage of the highest expressing tissue (newborn forebrain for *MYCN* and newborn thymus for *MYC*), which was arbitrarily set to 100%. (Panel A based on Hartl et al. 2010. Panel C based on data from Zimmerman et al. 1986.)

What evidence suggests that MYCN is truly distinct from MYC? In *MYCN* null embryos, the regional morphology of the central nervous system (hindbrain prominently) differed from wild-type embryos, despite up-regulation of *MYC* (Stanton et al. 1992). In addition to a lack of compensation, this observation suggests that *MYCN* and *MYC* may regulate each other's expression levels, as observed in other studies (Breit and Schwab 1989; Rosenbaum et al. 1989; Westermann et al. 2008; Helland et al. 2011). Although *MYCN* knockin can compensate for knockout of *MYC*, knockout of either *MYC* or *MYCN* results in embryonic lethality at approximately E10.5–E11.5 (Charron et al. 1992; Davis et al. 1993; Sawai et al. 1993). The inability of endogenous *MYCN* and *MYC* to compensate for these knockout phenotypes may be because of the distinct spatiotemporal expression patterns displayed by MYC family proteins (Fig. 1C).

Expression of *MYCN* is tissue specific, and is found during early developmental stages, whereas expression of *MYC* is more generalized (Fig. 1C). For example, expression of *MYCN* is highest in forebrain, kidney, and hindbrain of newborn mice, and is virtually absent in all tissues of adult mice. In contrast, expression of *MYC* was detected in a broad spectrum of tissues in newborn mice (highest in thymus, spleen, and liver), and subsided substantially in many, but not all, tissues of adult mice (adrenal and thymus maintained high levels; Zimmerman et al. 1986). The differential expression of *MYCN* and *MYC* is particularly striking in the kidneys and B-cell development, in which both *MYCN* and *MYC* are expressed before maturation, with *MYC* alone remaining detectable in kidneys and B cells in adult organisms (Zimmerman et al. 1986). Thus, double knockout of *MYCN* and *MYC* in hematopoietic cells has a significantly more disabling phenotype than either single knockout, suggesting cooperation in the biology of hematopoietic stem cells (Laurenti et al. 2008). Another structure demonstrating differential requirements for *MYCN* and *MYC* is the cerebellum. Conditional deletion of *MYCN* in neural stem and progenitor cells markedly reduced proliferation of cerebellar granule

neural precursors (GNPs); however, this effect was not seen with deletion of *MYC* (Hatton et al. 2006). This differential requirement for *MYCN* over *MYC* is further highlighted by the fact that sonic hedgehog (SHH) signaling drives the expansion of cerebellar GNPs and associates with transcription of *MYCN*, but not *MYC* (Kennedy et al. 2003).

Expression of *MYCN* is generally highest in immature cells in newborn mice, with reduced expression in differentiated adult tissues. Consistent with this paradigm is the finding that differentiation of neuroblastoma cells is associated with reduced expression of *MYCN* (Matsumoto et al. 1989; Cinatl et al. 1993; Han et al. 2001; Reddy et al. 2006). Interestingly, *MYCN* is down-regulated during retinoic acid-induced differentiation of neuroblastoma lines before cell cycle and morphological changes (Thiele et al. 1985). Conversely, *MYCN* has a direct role in blocking differentiation pathways and maintaining pluripotency (Wakamatsu et al. 1997; Kang et al. 2006; Nara et al. 2007; Cotterman and Knoepfler 2009; Lovén et al. 2010; Henriksen et al. 2011). In particular, mice with conditional deletion of *MYCN* in neural progenitor cells showed decreased brain size (especially in the cerebellum where its size was reduced six-fold) and showed a substantial increase in neuronal differentiation compared to control mice (Knoepfler et al. 2002). The enhanced differentiation in *MYCN* null neural progenitor cells may be a result of increased levels of the cyclin-dependent kinase inhibitor p27Kip1, which plays a role in differentiation and is normally degraded by the E3 ubiquitin ligase S-phase kinase associated protein (SKP2), a *MYCN* transcriptional target (Casaccia-Bonnel et al. 1997; Gómez-Casares et al. 2013). While *MYCN* and *MYC* were both found to have roles in maintaining pluripotency and self-renewal of stem cells, and both proteins can reprogram fibroblasts into induced pluripotent stem (iPS) cells (Nakagawa et al. 2010; Varlakhanova et al. 2010), evidence for differential functions of *MYC* and *MYCN* in regulating pluripotency comes from studies in medulloblastoma (see Roussel and Robinson 2013). When misexpressed in GNPs dependent on SHH signaling for survival,

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MYCN promotes an expected SHH-driven malignancy, whereas MYC drives malignancy down a distinct SHH-independent lineage. These data suggest that MYCN can transform cells in a committed lineage. In contrast, MYC can similarly transform cells, and can also drive cancer stem cell functionality, the latter enabling reprogramming down a distinct lineage (Kawauchi et al. 2012; Pei et al. 2012). It is likely that MYCN can also direct cancer stem cell functionality, through interactions with cooperating transcription factors. Support for this view comes from experiments in which co-expression of SOX9 with MYCN in cerebellar stem and progenitor cells could drive self-renewal, whereas neither SOX9 nor MYCN could drive self-renewal individually (Swartling et al. 2012).

MYCN IN NEUROBLASTOMA

Within two years of *MYCN*'s discovery in neuroblastoma, amplification of *MYCN* was shown to correlate with poor prognosis in patients (Brodeur et al. 1984; Seeger et al. 1985), a biomarker that is still used today to stratify risk. Mice with targeted misexpression of *MYCN* to the peripheral neural crest via the rat tyrosine hydroxylase (TH)-promoter developed neuroblastoma, establishing that misexpression of *MYCN* in migrating neural crest cells can initiate this disease (Weiss et al. 1997). Tumors in these mice had a prolonged latency and showed recurrent chromosomal copy number abnormalities, suggesting that genetic mutations in addition to misexpressed *MYCN* were required to promote neuroblast transformation. This requirement for additional mutations was supported by the fact that loss of tumor suppressors neurofibromin1 or retinoblastoma1 (Rb), when combined with misexpression of *MYCN*, resulted in reduced latency and increased penetrance for tumors. The sections below illustrate some of the many roles that MYCN subserves in neuroblastoma tumorigenesis (Fig. 2).

Metastasis

Metastasis occurs in ~50% of neuroblastoma patients at diagnosis (Maris et al. 2007) with

frequent spread to bone marrow (70%), bone (55%), lymph nodes (30%), liver (30%), and brain (18%) (DuBois et al. 1999). Not surprisingly, levels of *MYCN* correlate with invasive and metastatic behavior (Zaizen et al. 1993; Bénard 1995; Goodman et al. 1997). *MYCN* contributes to all facets of metastasis: adhesion, motility, invasion, and degradation of surrounding matrices. Specifically, *MYCN*-directed down-regulation of integrins $\alpha 1$ and $\beta 1$ promotes detachment from the extracellular matrix and allow cells to migrate and invade (van Golen et al. 2003; Tanaka and Fukuzawa 2008). *MYCN* promotes transcription of focal adhesion kinase (FAK), a critical regulator of integrin signaling, and generally promotes increased migration and metastasis in tumor cells (Beierle et al. 2007; Megison et al. 2012). Notably, FAK can also be repressed transcriptionally by p53, indicating a possible competition between *MYCN* and p53 in regulating FAK levels (Golubovskaya et al. 2008). *MYCN* also increases activity of matrix metalloproteinases (MMPs). In SHEP neuroblastoma cells, expression of *BCL2* led to an increase in expression and secretion of MMP2, whereas co-expression of *MYCN* and *BCL2* suppressed the MMP2 antagonist, TIMP-2 (Noujaim et al. 2002). miR-9, a microRNA activated by *MYCN*, targets and suppresses E-cadherin, contributing to an epithelial to mesenchymal transition (EMT) (Ma et al. 2010).

Although caspase-8 has been associated mostly with promoting apoptosis, caspase-8 also has a paradoxical role in both promoting and inhibiting metastasis in neuroblastoma, depending on the cellular context. A significantly higher incidence of spontaneous metastasis was observed in primary neuroblastoma tumors without caspase-8, as compared to tumors expressing caspase-8, although the primary tumor sizes were similar. NB7 neuroblastoma lines with ectopic expression of caspase-8 eventually lost expression of caspase-8 at sites of dissemination (Stupack et al. 2006). The lack of caspase-8 triggered integrin-mediated death, a mechanism of apoptosis in cells that have detached from the extracellular matrix and unligated their integrins (Stupack et al. 2001). In the TH-*MYCN* mouse model of neuroblastoma, deletion of ca-

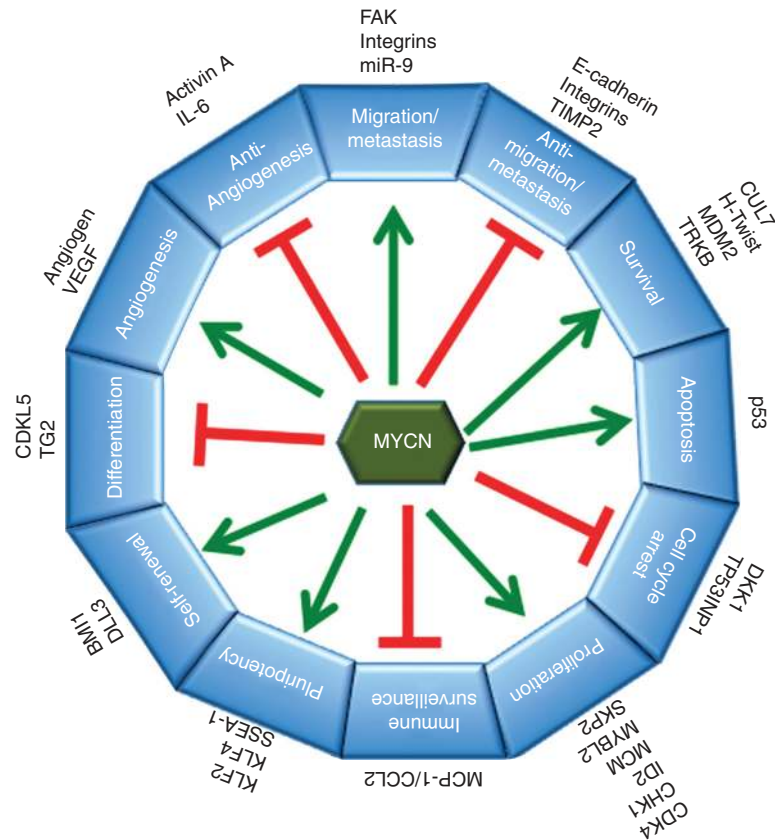


Figure 2. MYCN plays multiple roles in malignancy and maintenance of stem-like state. MYCN can activate transcription of genes involved in metastasis, survival, proliferation, pluripotency, self-renewal, and angiogenesis. Additionally, MYCN can suppress expression of genes that promote differentiation, cell cycle arrest, immune surveillance, and genes that antagonize metastasis and angiogenesis. Because MYCN also activates apoptotic pathways involving p53, MYCN is not normally sufficient for transformation.

pase-8 did not affect apoptosis, but instead, increased significantly the incidence of metastasis specifically to the bone marrow, possibly caused by up-regulation of genes involved in EMT, decreased cell adhesion, and increased fibrosis (Teitz et al. 2013). The prometastatic capabilities of caspase-8 were dependent on the tumor being resistant to apoptosis, which occurs when caspase-3 is lost. In this setting, caspase-8 associated with focal adhesion complex proteins, such as SRC, integrins, calpain-2 (CPN2), and FAK. Within this complex, caspase-8, independent of its proteolytic activity, permitted CPN2 to become activated and cleave focal adhesion substrates to promote cell migration (Barbero et al. 2009).

Immune Surveillance

MYCN influences immune surveillance by modulating antigens expressed on tumor cells. One antigen repressed by MYCN is monocyte chemoattractant protein-1/CC chemokine ligand 2 (MCP-1/CCL2), required for chemoattraction of natural killer T (NKT) cells (Song et al. 2007). Knockdown of MYCN in MYCN-amplified neuroblastoma lines rescued MCP-1 production and NKT cell chemoattraction. In contrast, overexpression of MYCN in neuroblastoma xenografts inhibited the ability to attract NKT cells. Presumably, MYCN binds to the E-box element of the MCP-1 promoter to block expression of the chemokine. Support for this model was ob-

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served in patients with *MYCN*-amplified neuroblastoma metastasizing to the bone marrow, which had fourfold fewer bone marrow NKT cells compared to their nonamplified counterparts (Song et al. 2007). Although NKT cells have not been shown directly to be effective against neuroblastoma cells, NKT cells can initially secrete a set of proinflammatory cytokines to recruit immune cells, stimulate the maturation of dendritic cells, and generate antigen-specific T cells to target the tumor.

Angiogenesis

High vascularity in neuroblastoma correlates with poor survival, increased dissemination, and amplification of *MYCN* (Meitar et al. 1996; Ribatti et al. 2002; Ozer et al. 2007). The association of vascularity with amplification of *MYCN* suggests antiangiogenic therapy as a viable approach towards *MYCN*-amplified neuroblastoma. Does *MYCN* facilitate the secretion of factors to promote growth of endothelial cells and/or block the release of inhibitors of angiogenesis? Conditioned media from neuroblastoma cell lines with induced expression of *MYCN* revealed the loss of inhibitors of endothelial growth (Fotsis et al. 1999). These inhibitors of angiogenesis include Activin A (Hatzi et al. 2000), leukemia inhibitory factor (LIF; Hatzi et al. 2002a), and interleukin 6 (IL-6; Hatzi et al. 2002b). Misexpression of *MYCN* also had a positive effect on proangiogenic factors including angiogenin (Dungwa et al. 2012) and vascular endothelial growth factor (VEGF) via the phosphoinositide 3'-kinase (PI3K)/mammalian target of rapamycin (mTOR) pathway (Kang et al. 2008). Indeed, the PI3K/mTOR inhibitor, NVP-BE235, suppressed growth of *MYCN*-amplified tumors and disrupted angiogenesis (Chanthery et al. 2012). Here, blockade of PI3K/mTOR signaling destabilized *MYCN*, which subsequently suppressed transcription and secretion of VEGF.

Self-Renewal and Pluripotency

As described above, neuroblastoma likely arises from neural crest cells, which possess the char-

acteristics of self-renewal and multipotency. *MYCN* is likely involved in the regulation of both traits, as *MYCN* can substitute for *MYC* in reprogramming fibroblasts into iPS cells (Nakagawa et al. 2010). Thus, both *MYC* and *MYCN* promote a stem-like state, likely because of blockade of differentiation pathways and expression of self-renewal and pluripotency factors. Indeed, *MYCN* up-regulates the pluripotency genes *KLF2*, *KLF4*, and *LIN28B* (Cotterman and Knoepfler 2009). Although *MYCN* also increased *LIF* expression in this study, another report found that *MYCN* reduced levels of *LIF* in neuroblastoma, suggesting that *MYCN* may differentially regulate *LIF* based on cellular context (Hatzi et al. 2002a). Mouse ESCs knocked out for both *MYC* and *MYCN* showed up-regulation of endodermal and mesodermal markers of differentiation (*BMP4*, *GATA6*), as well as activators of lymphocytic differentiation (*STAT1*, *EGR1*, *ELK3*), and sensory organ development (*DLL1*, *BMP4*, *GBX2*, *FGFR1*), suggesting that *MYC* proteins repress differentiation pathways (Varlakhanova et al. 2010). Other differentiation proteins suppressed by *MYCN* include cyclin-dependent kinase-like 5 (*CDKL5*) and tissue transglutaminase (*TG2*) (Liu et al. 2007; Valli et al. 2012). *MYC* and *MYCN* deleted mESC also lost expression of the pluripotency marker *SSEA-1*, although other pluripotency markers *NANOG*, *OCT4*, and *REX-1* were not affected significantly (Varlakhanova et al. 2010). Nevertheless, the loss of *MYC* and *MYCN* in mouse ESCs resulted in loss of self-renewal, in part, because of an inability to progress efficiently through S-phase and the G₂/M checkpoint. Importantly, *MYCN* up-regulates expression of the polycomb and self-renewal protein *BMI1* in neuroblastoma by directly binding to the E-Box sites within the promoter of *BMI1* (Ochiai et al. 2010; Huang et al. 2011). Additionally, *MYCN*-mediated expression of *DLL3*, a Notch1 ligand, has been described as a mechanism to maintain neural stem cell fate (Zhao et al. 2009). Further support for *MYCN* promoting self-renewal is the finding that *MYCN*-amplified neuroblastoma cell lines tend to undergo symmetric cell division, whereas nonamplified lines preferentially divide asymmetrically (Izumi and

Kaneko 2012). Thus, MYCN plays a critical role in maintaining a stem-like state by blocking differentiation pathways and activating self-renewal and pluripotency genes (see Chappell and Dalton 2013).

Apoptosis

Like MYC, MYCN activates both proliferation and apoptosis (Evan et al. 1992; Fulda et al. 2000). Whether MYCN promotes a net proliferative response is therefore dependent on the status of cooperating apoptotic factors, such as the antiapoptotic protein, BCL2 (Strasser et al.

1990) or p53 (Elson et al. 1995; Chesler et al. 2008). Interestingly, *TP53* mutations are rare in neuroblastoma at diagnosis, suggesting that MYCN likely cooperates with suppressors of p53 signaling, including miRNA-380-5p, the oncogene *CUL7*, *BMI1*, and transcription factor H-Twist, and MDM2 (Valesia-Wittmann et al. 2004; Slack et al. 2005; Kim et al. 2007; Swarbrick et al. 2010; Huang et al. 2011). In contrast, mutations in *TP53* and p53 pathway members occur commonly in neuroblastoma at relapse, consistent with the idea that such mutations, which drive therapy-resistant disease, may arise in response to cytotoxic chemotherapy. The impor-

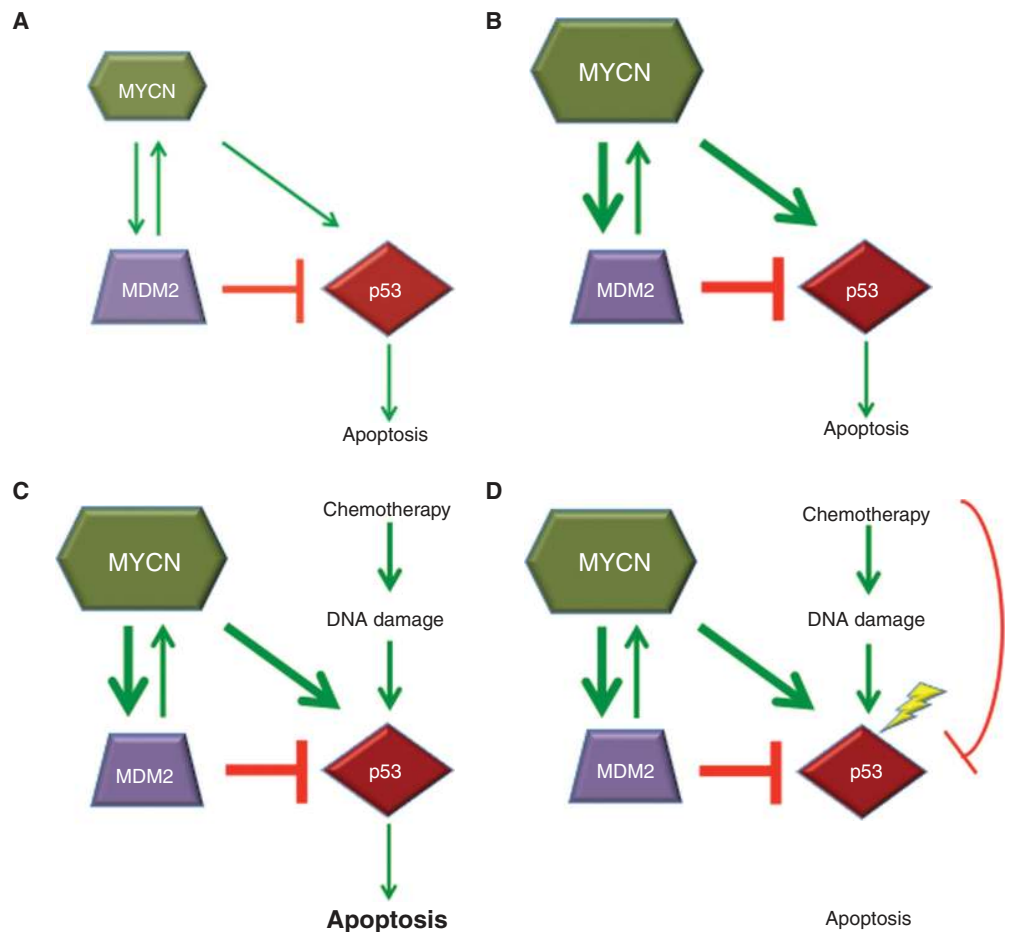


Figure 3. Amplified *MYCN* marks chemoresistance in patients with neuroblastoma. (A,B) Amplified *MYCN* results in elevated transcription of *MDM2* and *TP53*. High levels of p53 sensitize tumors to apoptosis. (C) Patients initially respond to chemotherapy as p53 triggers apoptosis. (D) At relapse, tumors show mutation in p53 or p53 pathways, resulting in therapy resistance.

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tance of p53 signaling is further highlighted by the role of MYCN in the MDM2-p53 pathway (Fig. 3). MDM2 is an E3 ubiquitin ligase that promotes survival by ubiquitinating and driving degradation of p53, and has also been implicated in promoting the stability of MYCN mRNA by binding to the AU-rich elements of the 3'UTR of MYCN (Gu et al. 2012). Interestingly, MDM2 is a target for p53-mediated transcription, and MYCN can promote transcription of both MDM2 and TP53 (Slack et al. 2005; Chen et al. 2010). The role of MYCN in activating both MDM2 and TP53 explains conflicting sensitivities to chemotherapy in MYCN-amplified neuroblastoma. MYCN-amplified neuroblastoma initially respond to chemotherapy, perhaps due, in part, to MYCN-mediated activation of p53. However, these tumors eventually relapse and become resistant, possibly through chemotherapy-induced inactivating mutations of p53, resulting in a feed-forward loop between MDM2 and MYCN to promote survival (Fig. 3).

Loss of caspase-8 function in neuroblastoma has been speculated to be a mechanism of apoptosis evasion in MYCN-amplified neuroblastoma (Teitz et al. 2000; Gonzalez-Gomez et al. 2003; Iolascon et al. 2003). A later study did not find correlation between caspase-8 expression and risk in neuroblastoma (MYCN status, chromosome 1p36 deletion, disease stage, etc.) (Fulda et al. 2006); however, multiple groups have reported methylation of the promoter for caspase-8 (Casciano et al. 2004; Banelli et al. 2005; Lázcóz et al. 2006). Loss of caspase-8 promotes resistance to tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis (Eggert et al. 2001).

In addition to associating with suppression of proapoptotic pathways, MYCN-amplified neuroblastoma could survive via constitutive activation of prosurvival signaling cascades, such as tropomyosin receptor kinase B (TRKB). Interestingly, levels of TRKB expression are low in neuroblastoma not amplified for MYCN. In contrast, TRKB is frequently activated in MYCN-amplified neuroblastoma, signaling through both autocrine and paracrine survival pathways (Nakagawara et al. 1994). Activation of TRKB is

implicated in resistance to chemotherapy and can up-regulate MYCN mRNA, which may provide insights into the association of TRKB with MYCN-amplified neuroblastoma (Ho et al. 2002; Dewitt et al. 2013). Conversely, MYCN appears to down-regulate tropomyosin receptor kinase A (TRKA), which is normally found in low-risk neuroblastoma, recruiting HDAC1 to the TRKA promoter to induce a repressed chromatin state (Iraci et al. 2011).

Proliferation and Cell Cycle

The best characterized tumorigenic effect of MYCN is to promote proliferation and cell cycle progression. Specifically, MYCN-amplified neuroblastomas show an inability to arrest in G₁-phase in response to irradiation and DNA damage, possibly via down-regulation of TP53 inducible nuclear protein 1 (TP53INP1) and up-regulation of both CDK4 and SKP2, allowing CDK2 to escape p21 inhibition (Tweddle et al. 2001; Bell et al. 2007; Muth et al. 2010; Gogolin et al. 2013). MYCN up-regulation of checkpoint kinase 1 (CHK1), an important regulator of S-phase and G₂/M checkpoints, has been suggested as a mechanism through which MYCN-amplified neuroblastoma becomes refractory to standard chemotherapy (Cole et al. 2011). Indeed, inhibition of CHK1 promotes chemosensitization in various types of tumor cells (Blasina et al. 2008; Zhang et al. 2009). MYCN also directly represses expression of anti-proliferative proteins such as Dickkopf-1, which disrupts the WNT/β-catenin signaling pathway, and CDKL5, which arrests cells between G₀/G₁ phase (Koppen et al. 2007a; Valli et al. 2012). ID2, a helix-loop-helix transcription factor, is also a target of MYCN. MYCN directly binds to the promoter of ID2 to stimulate its expression, leading to inactivation of Rb to permit progression through the cell cycle (Iavarone et al. 1994; Lasorella et al. 1996). Consistent with the idea that MYCN activates transcription of ID2, MYCN-amplified neuroblastoma lines show elevated levels of ID2, and MYCN and ID2 expression levels correlate during development (Lasorella et al. 2000; 2002).



MYC has also been implicated in progression through the G₁-S phase of the cell cycle by cooperating with RAS to induce cyclin D/CDK4 and/or cyclin E/CDK2 complexes that phosphorylate and inactivate Rb proteins, activating E2F transcription factors to induce S-phase (reviewed in Farrell and Sears 2014). This cycle is further amplified because RAS signaling stabilizes MYC proteins by activating AKT, which phosphorylates and blocks glycogen synthase kinase 3 β (GSK-3 β)-mediated degradation of MYC proteins. Thus, blockade of RAS signaling can induce growth arrest in *MYCN*-amplified neuroblastoma cells (Yaari et al. 2005). mTOR signals downstream from the RAS/PI3K/AKT pathway and has essential functions in translational control, impacting metabolism, proliferation, and tumorigenesis. Allosteric blockade of mTOR with rapamycin or CCI-779 displayed a more pronounced antiproliferative effect in *MYCN*-amplified than in *MYCN*-nonamplified neuroblastoma tumors. Additionally, allosteric mTOR inhibitors also suppressed expression of VEGF-A and cyclin D1 (Johnsen et al. 2008). Other targets of MYCN that appear to drive proliferation are neuronal leucine-rich repeat protein-1, which also enhances expression of *MYCN*, the transcription factor MYBL2, a downstream target of MYCN and a factor in drug resistance, and minichromosome maintenance (MCM) genes that are responsible for DNA elongation and unwinding during the replication process (Raschella et al. 1999; Koppen et al. 2007b; Hossain et al. 2008; 2012; Gualdrini et al. 2010).

EPIGENETIC ROLES OF MYCN

Because neuroblastoma typically occurs in early childhood (as opposed to adult cancers that have more time to accumulate mutations that promote transformation), the profound tumorigenic influence of MYCN likely extends beyond its ability to directly regulate expression of any individual gene. MYCN can also indirectly mediate the expression of multiple genes simultaneously via activation of noncoding RNAs, such as miRNA and long noncoding RNA (reviewed in Buechner and Einvik 2012). Additional-

ly, MYCN impacts global histone methylation and acetylation, markers of transcriptional repression and activation, respectively (Knoepfler et al. 2006). Recently, MYCN was found to associate with EZH2, a methyltransferase and member of the polycomb repressor complex 2 (Corvetta et al. 2013). EZH2 has been implicated in the trimethylation of Histone 3 K27, a transcriptional silencing mark (Kotake et al. 2007; Au et al. 2012). Interestingly, the interaction between MYCN and EZH2 requires the MYC box domain III, which is necessary for MYC to promote transformation (Herbst et al. 2005). In support of a transcriptionally repressive function for a MYCN/EZH2 complex is the finding that the interaction between MYC and another DNA methyltransferase, DNMT3A, silences the transcription of p21CIP1, an inhibitor of cyclin-dependent kinases (Brenner et al. 2005). Furthermore, MYCN up-regulates the expression of histone deacetylases (HDACs) in neuroblastoma, including HDAC1, HDAC2, and SIRT1. HDAC1 represses expression of the differentiation protein TG2. HDAC2 silences cyclin G2, a suppressor of cell cycle progression, whereas SIRT1 represses mitogen-activated protein kinase phosphatase 3, activating ERK (Marshall et al. 2010; 2011; Iraci et al. 2011). Notably, SIRT1 stabilizes MYCN, promoting a feed-forward loop between the two proteins (Marshall et al. 2011). Thus, MYCN may enhance silencing of tumor suppressors in neuroblastoma by increasing DNA methylation and deacetylation.

ROLE OF MYCL AND MYC IN NEUROBLASTOMA

Amplification of other MYC family members (i.e., MYCL, MYC) is uncommon in neuroblastoma (Slavc et al. 1990). As compared with levels of *MYCL* and *MYC*, expression of *MYCN* is higher in developing tissues that normally give rise to neuroblastoma (Kohl et al. 1986; Stanton et al. 1992). In fact, loss of heterozygosity for chromosome 1p (a marker of risk) suggests that deletion of *MYCL* (on chromosome 1p34) may indicate unfavorable prognosis (Hiyama et al. 2001), with the caveat that other tumor suppressors also reside in this subchromosomal

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region. *MYC* is infrequently amplified in neuroblastoma, and expression of *MYC* correlates inversely with *MYCN* (Breit and Schwab 1989). Specifically, *MYCN*-nonamplified Stage 4 and 4S neuroblastoma tumors may show high levels of *MYC* mRNA (Westermann et al. 2008), suggesting that amplified *MYC* may compensate in *MYCN*-nonamplified tumors.

TARGETING *MYCN*-AMPLIFIED NEUROBLASTOMA

Knockdown of *MYCN* expression via RNA interference and antisense oligonucleotides in *MYCN*-amplified neuroblastoma has shown an increase in apoptosis and differentiation,

and suppression of cell growth, suggesting that blockade of *MYCN* may be a therapeutic option in *MYCN*-driven neuroblastoma (Burkhardt et al. 2003; Kang et al. 2006). However, the development of inhibitors targeting *MYC* proteins has been challenging, as *MYC*/*MYCN* proteins are composed of two extended alpha-helices with no obvious surfaces for small molecule binding. Strategies to circumvent blocking *MYCN* directly include: (1) targeting epigenetic reader proteins, such as acetyl-lysine binding modules (bromodomains) that link chromatin marks to activation of *MYC*/*MYCN*, (2) targeting regulators of *MYCN* mRNA and protein stability, (3) activating p53-induced apoptosis, and (4) triggering differentiation (Fig. 4).

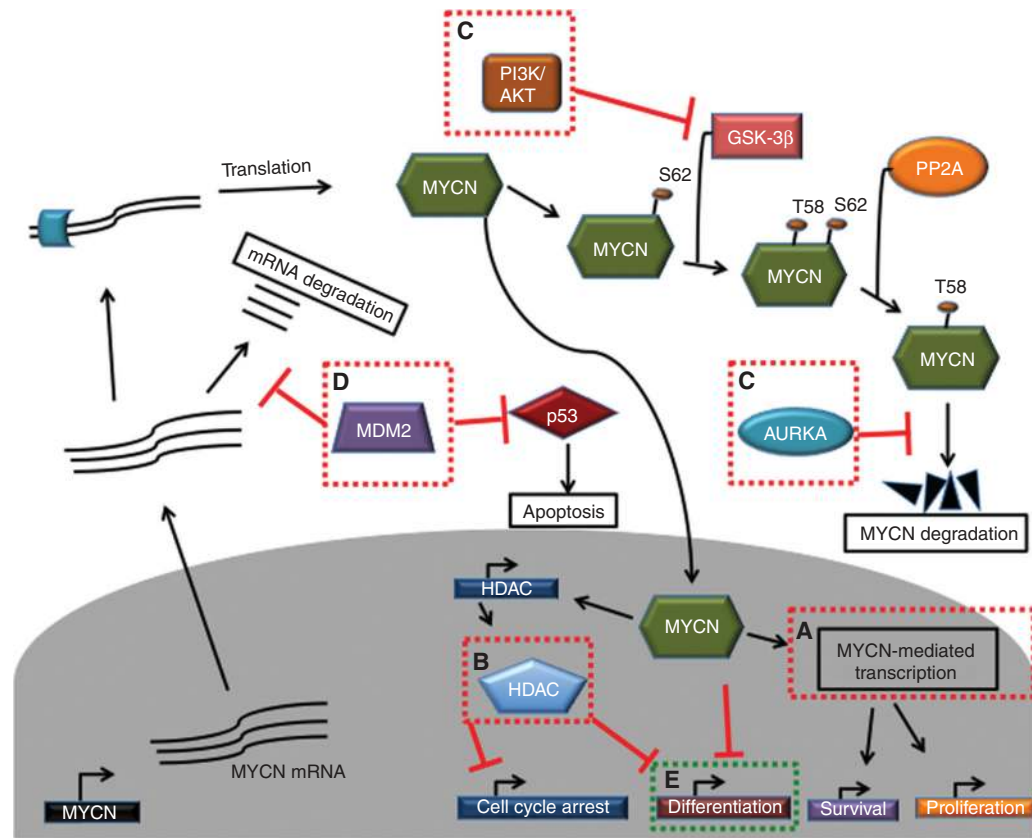


Figure 4. Therapeutic strategies to target *MYCN* in neuroblastoma. Possible strategies to treat *MYCN*-amplified neuroblastoma patients include (A) blocking *MYCN*-dependent transcription with BET-bromodomain inhibitors, (B) inhibiting HDACs, (C) antagonizing proteins involved in stabilizing *MYCN* protein, (D) suppressing MDM2 (which stabilizes *MYCN* mRNA and disrupts p53-mediated apoptosis), and (E) inducing differentiation.



BET Bromodomain Inhibitors

Gene expression from enhancer and promoter-bound oncogenic transcription factors is mediated by multiprotein assemblies, such as the mediator complex, the positive transcriptional elongation factor complex (PTEFB), and chromatin-associated protein complexes and chromatin-remodeling complexes (e.g., SWI/SNF). In addition, memory of the cancer-cell state is maintained through cell division by covalent modification of the unstructured amino-terminal tails of histone proteins (see Bradner 2013; Sabó and Amati 2014). Lysine side-chain acetylation figures prominently in activation of MYCN. Acetylation marks are placed in regions of active transcription by histone acetyltransferases. Their interpretation is mediated by acetyllysine reader domains or bromodomains, a family of 47 transcriptional coactivators. Members of the bromodomain and extraterminal (BET) subfamily, BRD2-4, are compelling targets owing to characterized binding interactions with SWI/SNF and their interaction with PTEFB. MYC also recruits PTEFB to release RNA polymerase II, promoting transcription. The tool compound and BRD2-4 inhibitor JQ1 was shown to block MYC targets as well as MYC itself in multiple myeloma, a MYC-dependent malignancy (Delmore et al. 2011). JQ1 also suppresses growth of MYCN-amplified neuroblastoma in multiple *in vivo* models including orthotopic transplantation of patient derived xenografts and the TH-MYCN mouse model. Mice treated with JQ1 showed a significant increase in overall survival, and tumors showed increased apoptosis and decreased proliferation as well as decreased expression of MYCN itself (Puissant et al. 2013). Thus, BET bromodomain inhibitors may be a therapeutic option for patients with MYCN-amplified neuroblastoma (Fig. 4A).

HDAC

As described above, MYCN silences tumor suppressor genes by recruitment of DNA methyltransferases and elevated expression of HDAC, suggesting a possible therapeutic role for HDAC inhibitors. Several preclinical studies have found

promising results using HDAC inhibitors in the TH-MYCN model of neuroblastoma. For instance, treatment of TH-MYCN mice with the SIRT1 inhibitor, Cambinol, reduced tumorigenesis (Marshall et al. 2011). Liu and colleagues found that Trichostatin A (class I and II HDAC inhibitor) restored expression of the differentiation protein TG2, resulting in reduced tumor weight and volume in the same mouse model (Liu et al. 2007). Therefore, HDAC inhibitors may be a viable route to target MYCN-amplified neuroblastoma (Fig. 4B).

Regulators of MYC Protein Stability

MYC and MYCN proteins are proteolyzed through a sequential stepwise set of phosphorylation events. Initially, MYC and MYCN are phosphorylated at S62 (via kinases in the RAS signaling pathway, such as MAPK [Seth et al. 1991; Lutterbach and Hann 1994] and CDK1 [Sjostrom et al. 2005]), stabilizing MYC and MYCN and priming these proteins for phosphorylation at T58 via GSK-3 β (Pulverer et al. 1994). Dephosphorylation of S62 via protein phosphatase 2A (PP2A) sensitizes MYC and MYCN phosphorylated at T58 to bind F-box and WD repeat domain-containing 7 (FBW7) or other E3 ligases, leading to the ubiquitination and degradation in the proteasome (Fig. 4) (Sears et al. 2000; Welcker et al. 2004; Yada et al. 2004).

PI3K inhibition can decrease MYCN protein levels without impacting the mRNA by blocking a PI3K-driven inhibitory phosphorylation on GSK-3 β and promoting MYCN phosphorylation (Fig. 4C) (reviewed in Gustafson and Weiss 2010). Neuroblastoma lines expressing MYCN^{T58A} (lacking a GSK-3 β phosphorylation site) were resistant to antiproliferative effects from treatment with the PI3K/mTOR inhibitor, LY294002 (Chesler et al. 2006). These data, and subsequent studies using the clinical inhibitor BEZ235 (Chanthery et al. 2012) suggest that PI3K/mTOR inhibitors represent a viable strategy to target MYCN-amplified neuroblastoma tumors.

Proteins that prevent dephosphorylation at T58 stabilize MYCN, which may be the case

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with aurora kinase A (AURKA) in neuroblastoma (Fig. 4C) (Otto et al. 2009). Interestingly, increased expression of AURKA is found in *MYCN*-amplified neuroblastoma, mediated potentially by *MYCN* itself (Shang et al. 2009). Thus, elevated levels of either *MYCN* or AURKA promote a potential feed-forward loop that stabilizes both proteins. Because AURKA has possible ligand binding sites for small molecule inhibitors, targeting AURKA could potentially represent a strategy to treat *MYCN*-amplified tumors (Faisal et al. 2011); however, the ability of AURKA to stabilize *MYCN* has been shown to be independent of kinase activity (Otto et al. 2009). A similar mechanism of tumor suppression has also been proposed for protein tyrosine phosphatase receptor type D (PTPRD). Ectopic expression of PTPRD dephosphorylates tyrosine residues on AURKA, which destabilizes AURKA, and subsequently lowers levels of *MYCN* and proliferation (Meehan et al. 2012).

MDM2

Down-regulation of MDM2 would presumably have two significant effects in *MYCN*-amplified neuroblastoma. Because MDM2 can bind to the 3'UTR of *MYCN*, loss of MDM2 would destabilize *MYCN* mRNA and subsequently block expression of *MYCN* protein (Gu et al. 2012). In parallel, decreased MDM2 levels should stabilize p53 and increase the likelihood of p53-depend

ent apoptosis (Fig. 4D). Indeed, *MYCN*-amplified neuroblastoma cell lines have been found to be more sensitive than *MYCN*-nonamplified lines to antagonists of MDM2: Nutlin-3 and MI-63 (Gamble et al. 2012).

Inducers of Differentiation

Because neuroblastoma is believed to originate from immature neural crest cells, triggering differentiation in these cells should result in reduced proliferation and increased cell death. Indeed, retinoic acid, nitric oxide, and phenylacetate have all been shown to induce differentiation, and inhibit both anchorage independence and cell growth in neuroblastoma (Sidell 1982; Han et al. 2001; Ciani et al. 2004). Although *MYCN* is known to inhibit differentiation pathways, each of these molecules have been found to reduce *MYCN* levels as well, indicating that *MYCN*-amplified tumors can still be induced to differentiate as a means to suppress proliferation and promote apoptosis (Fig. 4E).

OTHER GENETIC DRIVER MUTATIONS OF NEUROBLASTOMA

To identify novel driver mutations of neuroblastoma, intense efforts have focused on genome-wide association studies (GWAS), as well as whole exome and whole genome sequencing studies of tumor samples (Table 1). Although

Table 1. Non-*MYCN* drivers of neuroblastoma

Gene	GOF or LOF	Frequency	Number of tumors
<i>ALK</i>	GOF	6%, ^{1,2} 8%, ³ 9%, ⁴ 12.4%, ⁵ 14%, ⁶	87, ¹ 215, ² 93, ³ 240, ⁴ 194, ⁵ 130 ⁶
<i>ATRX</i>	LOF	9.6%, ⁴ 25% ^{7,a}	240, ⁴ 40 ⁷
<i>ARID1A</i>	LOF	1%–2%, ⁴ 6% ⁶	240, ⁴ 71 ⁶
<i>ARID1B</i>	LOF	<1%, ⁴ 7% ⁶	240, ⁴ 71 ⁶
<i>LIN28B</i>	GOF	1.1%, ⁸ 1.4% ⁶	263, ⁸ 71 ⁶
<i>PTPN11</i>	GOF	2.9%, ⁴ 3.4% ⁹	240, ⁴ 89 ⁹
<i>TIAM1</i>	LOF	0%, ⁴ 3% ¹	240, ⁴ 87 ¹

Tumor susceptibility SNPs were identified in *BARD1* (Capasso et al. 2009; Nguyen et al. 2011; Diskin et al. 2012), *HACE1* (Diskin et al. 2012), *LMO1* (Wang et al. 2011; Diskin et al. 2012; Nguyen et al. 2011), and *LIN28B* (Diskin et al. 2012). Pugh et al. (2013) identified other genetic mutations that had frequencies <1%.

References: ¹Molenaar et al. 2012b, ²Chen et al. 2008, ³George et al. 2008, ⁴Pugh et al. 2013, ⁵Mossé et al. 2008, ⁶Sausen et al. 2013, ⁷Cheung et al. 2012, ⁸Molenaar et al. 2012a, ⁹Bentires-Alj et al. 2004.

^aHalf of tumors with *ATRX* mutations were from older patients, which account for a small percentage of neuroblastoma patients.

subchromosomal aberrations occur robustly in this disease, genetic mutations were quite rare (some could not be verified in independent studies). Two of the more commonly altered genes that have been observed in multiple studies include anaplastic lymphoma kinase (*ALK*) and Alpha thalassemia/mental retardation syndrome X-linked (*ATRX*).

ALK

Familial neuroblastoma occurs rarely compared to sporadic cases. Gain of function mutations in the receptor tyrosine kinase *ALK* (chromosome 2p23) occur in ~50% of familial neuroblastoma cases, and ~7% of sporadic neuroblastoma cases (Chen et al. 2008; George et al. 2008; Janoueix-Lerosey et al. 2008; Mossé et al. 2008; Molenaar et al. 2012b). *ALK* can drive neuroblastoma in genetically engineered mice misexpressing the most potent mutation of *ALK*, F1174L (Heukamp et al. 2012; Schulte et al. 2012). Additionally, *ALK* cooperates with *MYCN* to drive malignancy, as activation of *ALK* results in increased expression of *MYCN* by elevating activity of the *MYCN* promoter, and stabilizing *MYCN* protein likely via activation of *AKT* and *ERK* (Chesler et al. 2006; Berry et al. 2012; Schönherr et al. 2012; Zhu et al. 2012). *ALK* and *MYCN* are also physically linked on chromosome 2p, which may explain why amplification of *MYCN* and *ALK* frequently co-occur (De Brouwer et al. 2010).

ATRX

Whole genome sequencing of 40 neuroblastoma patient samples revealed loss of function mutations in *ATRX* (chromosome Xq21) in 10 samples, five of which belong to older patients, which constitutes a small percentage of overall neuroblastoma cases. Eight of the 10 samples with *ATRX* mutations showed longer telomeres (via alternative lengthening of telomeres [ALT]), and lacked *ATRX* in the nucleus (Cheung et al. 2012). Patients with *ATRX* mutant neuroblastoma were typically adolescents with a chronic progressive form of this disease. Other than telomere lengthening, *ATRX* is thought to function

epigenetically by recruitment of Histone 3.3 at telomeric regions to maintain chromosome stability (reviewed in De La Fuente et al. 2011). Intriguingly, *ATRX* mutations were mutually exclusive from amplification of *MYCN* in neuroblastoma, which was verified in a separate study (Pugh et al. 2013). In contrast, elevated expression of *MYCN* and mutation of *ATRX* are both associated with mutation of G34 site in Histone 3.3 in pediatric glioblastoma (Schwartzentruber et al. 2012; Wu et al. 2012; Bjerke et al. 2013).

In a separate study, a subset of neuroblastoma tumors (five of 87) with *ATRX* mutations also had defects in *PTPRD* and odd Oz 3 (*ODZ3*) (Molenaar et al. 2012b). The investigators reasoned that these three genes were likely co-selected, as the probability of finding defects in three concurrent genes in at least five tumors is $< 10^{-4}$. The connection among these genes may relate to a role in neuritogenesis, which is critical for differentiation of neuroblasts. *PTPRD* and *ODZ3* are transmembrane receptors that localize to axons and axonal growth cones and are known to enhance neuritogenesis (Arregui et al. 2000). Transgenic mice with *ATRX* mutations showed aberrant dendritic spine morphology and altered signaling through the GTPase, *Rac1*, which also promotes neuritogenesis (Shioda et al. 2011). Importantly, activators of *Rac1* (guanine exchange factors [GEF]), but not inhibitors (GTPase activating proteins [GAP]) are mutated in neuroblastoma, albeit rarely (e.g., *TIAM1* was found mutated in three out of 87 tumors) (Molenaar et al. 2012b). In contrast, *Rho* signaling opposes neuritogenesis and more mutations in *Rho* GAPs than GEFs were identified in the same study (Molenaar et al. 2012b). Taken together, this evidence implicates neuritogenesis as a process that is antagonized in neuroblastoma tumorigenesis. Interestingly, a recent study by Pugh et al. (2013) did not detect mutations in GEFs and GAPs involved in *Rac/Rho* signaling to a statistically significant degree, with *TIAM1*, the *Rac1* GEF most frequently mutated in the Molenaar study, not altered in the larger study by Pugh et al. (2013). The discrepancy between these studies may be a result of the rarity of neuritogenesis mutations and/or biological differences between the two data sets.

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Recently, a correlation was described among different types of tumors with respect to the frequency of mutations within the telomerase reverse transcriptase (*TERT*) promoter, with neural tumors (e.g., glioblastoma, oligodendroglioma, and medulloblastoma), particularly in older patients, showing the highest occurrences (Killela et al. 2013). These mutations are linked to maintaining telomere length, analogous to the effect of ALT, a commonly occurring phenomenon due to *ATRX* mutations. Based on these data, the Yan group hypothesizes that tumor types showing high frequencies of ALT might also show high frequencies of *TERT* mutations, and these mutations, as exemplified by glioblastoma, would be distributed in a mutually exclusive fashion. Older children with neuroblastoma have been shown to have *ATRX* mutations. To our knowledge, however, *TERT* mutations were not examined in these sequencing studies, an intriguing area for future investigation.

Remarks

The search for driver mutations in neuroblastoma has led to the identification of several other candidate genes, although the frequencies of mutations among these candidates are much lower than *ALK* and *ATRX*. These include gain of function in protein tyrosine phosphatase 11 (*PTPN11*) and *LIN28B*, structural mutations in neural growth cone genes (*ODZ3*, *PTPRD*, and *CSMD1*), and heterozygous loss of AT-rich interactive domain 1A and 1B (*ARID1A* and *ARID1B*; Molenaar et al. 2012a,b; Pugh et al. 2013; Sausen et al. 2013). Tumor susceptibility SNPs were associated with loci located at or near BRCA1-associated RING domain-1 (*BARD1*), HECT domain- and ankyrin repeat-containing E3 ubiquitin protein ligase 1 (*HACE1*), *LIN28B*, and LIM domain only 1 (*LMO1*) (Capasso et al. 2009, 2013; Wang et al. 2011; Diskin et al. 2012). In addition to the low mutation frequencies, several of these genes were not validated in multiple studies (e.g., *PTPRD*, *ODZ3*, *CSMD1*, *TIAM1*). In fact, while one study found *PTPRD* acts as a tumor suppressor in neuroblastoma (Meehan et al. 2012), a separate study found overexpression of *PTPRD* to have minimal effect

on colony formation in neuroblastoma cells, whereas levels of *PTPRD* were similar between embryonic adrenal cells and neuroblastoma lines, calling into question the role of *PTPRD* in neuroblastoma (Clark et al. 2012). One of the largest studies to date (240 samples) identified additional mutations (including a recurrent point mutation in *MYCN* (P44L) at a frequency of ~2%, leading to a twofold increased level of expression); however, the vast majority of mutations occurred at frequencies under 1% (Pugh et al. 2013).

The low mutation rate in neuroblastoma resembles other pediatric tumors, including medulloblastoma (Pugh et al. 2012), and stands in contrast to changes in chromosomal copy number, which are quite frequent and robust. Further support for the idea that chromosome aberrations contribute to neuroblastoma is the finding that chromothripsis (shredding of subchromosomal regions and random reassembly of fragments) may be associated with high-risk and late-stage neuroblastoma, as this phenomenon was observed in one of the recent papers reporting sequencing results (Molenaar et al. 2012b). Might neuroblastoma be driven by these subchromosomal gains and losses? For example, deletion of chromosome 1p or 11q occurs in 25%–35% of patients, whereas gain of chromosome 17q occurs in >70% of tumors (Plantaz et al. 1997; Attiyeh et al. 2005; Mossé et al. 2007). Interestingly, multiple studies have found amplification of *MYCN* to be directly correlated with chromosome 1p loss, while inversely related to chromosome 11q deletion (Cheng et al. 1995; Komuro et al. 1998; Caron et al. 2001; Attiyeh et al. 2005; Carén et al. 2010). In addition, gains of chromosome 17q have also been detected in tumors with amplified *MYCN*, although this is not unexpected because of the high frequency of chromosome 17q gains (Plantaz et al. 1997; Valentijn et al. 2005). Potentially, these somatic copy number variations of subchromosomal regions could equate to multiple genetic mutations occurring simultaneously, contributing to transformation. Modeling these chromosomal abnormalities, and identifying minimal regions of gains and losses may lead to the identification of new and potent drivers of neuroblastoma.



SUMMARY

Although neuroblastoma was first described more than 100 years ago, progress in identifying genetic causes and therapeutic targets has taken time. In 1983, *MYCN* was identified as the second member of the *MYC* family and two years later, amplification of *MYCN* was identified as among the first genetic biomarkers of any cancer, specifically marking high-risk neuroblastoma (Brodeur et al. 1984; Seeger et al. 1985). A genetically engineered mouse model of neuroblastoma (TH-*MYCN*) was established in 1997 and along with other studies, implicated *MYCN* in all aspects of tumorigenesis and malignancy (Nakagawara et al. 1994; Weiss et al. 1997; Song et al. 2007; Kang et al. 2008; Cotterman and Knoepfler 2009; Ma et al. 2010). Unfortunately, because *MYCN* is considered “undruggable,” standard care for *MYCN*-amplified neuroblastoma patients does not include targeting *MYCN* itself. GWAS, whole exome, and whole genome sequencing studies over the past few years have identified few recurrently mutated genes. *ALK* represents one of the most druggable and promising targets and was validated as a driver of neuroblastoma in both fish and mouse models (Heukamp et al. 2012; Schulte et al. 2012; Zhu et al. 2012). However, *ALK* mutations occur in <10% of neuroblastoma cases and initial clinical studies using targeted therapy against *ALK* in these patients have been disappointing (Carpenter and Mossé 2012). Preclinical studies using bromodomain inhibitors and targeting molecules that destabilize *MYCN* show promise preclinically, and should be tested in children with aggressive forms of neuroblastoma. In addition, because chromosomal aberrations appear to be robust in pediatric tumors, clues for uncovering novel drivers and therapeutic targets may depend on developing models of these chromosomal abnormalities.

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REFERENCES

*Reference is also in this collection.

- Akter J, Takatori A, Hossain MS, Ozaki T, Nakazawa A, Ohira M, Suenaga Y, Nakagawara A. 2011. Expression of *NLR3* orphan receptor gene is negatively regulated by *MYCN* and *Miz-1*, and its downregulation is associated with unfavorable outcome in neuroblastoma. *Clin Cancer Res* **17**: 6681–6692.
- Arregui CO, Balsamo J, Lilien J. 2000. Regulation of signaling by protein-tyrosine phosphatases: Potential roles in the nervous system. *Neurochem Res* **25**: 95–105.
- Attiyeh EF, London WB, Mossé YP, Wang Q, Winter C, Khazi D, McGrady PW, Seeger RC, Look AT, Shimada H, et al. 2005. Chromosome 1p and 11q deletions and outcome in neuroblastoma. *N Engl J Med* **353**: 2243–2253.
- Au SL-K, Wong CC-L, Lee JM-F, Fan DN-Y, Tsang FH, Ng IO-L, Wong C-M. 2012. Enhancer of zeste homolog 2 epigenetically silences multiple tumor suppressor microRNAs to promote liver cancer metastasis. *Hepatology* **56**: 622–631.
- Banelli B, Gelvi I, Di Vinci A, Scaruffi P, Casciano I, Allemani G, Bonassi S, Tonini GP, Romani M. 2005. Distinct CpG methylation profiles characterize different clinical groups of neuroblastic tumors. *Oncogene* **24**: 5619–5628.
- Barbero S, Mielgo A, Torres V, Teitz T, Shields DJ, Mikolon D, Bogyo M, Barilà D, Lahti JM, Schlaepfer D, et al. 2009. Caspase-8 association with the focal adhesion complex promotes tumor cell migration and metastasis. *Cancer Res* **69**: 3755–3763.
- Beierle EA, Trujillo A, Nagaram A, Kurenova EV, Finch R, Ma X, Vella J, Cance WG, Golubovskaya VM. 2007. N-MYC regulates focal adhesion kinase expression in human neuroblastoma. *J Biol Chem* **282**: 12503–12516.
- Bell E, Lunec J, Tweddle DA. 2007. Cell cycle regulation targets of *MYCN* identified by gene expression microarrays. *Cell Cycle* **6**: 1249–1256.
- Bénard J. 1995. Genetic alterations associated with metastatic dissemination and chemoresistance in neuroblastoma. *Eur J Cancer* **31A**: 560–564.
- Bentires-Alj M, Paez JG, David FS, Keilhack H, Halmos B, Naoki K, Maris JM, Richardson A, Bardelli A, Sugarbaker DJ, et al. 2004. Activating mutations of the Noonan syndrome-associated *SHP2/PTPN11* gene in human solid

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- tumors and adult acute myelogenous leukemia. *Cancer Res* **64**: 8816–8820.
- Berry T, Luther W, Bhatnagar N, Jamin Y, Poon E, Sanda T, Pei D, Sharma B, Vetharoy WR, Hallsworth A, et al. 2012. The *ALK^{F1174L}* mutation potentiates the oncogenic activity of MYCN in neuroblastoma. *Cancer Cell* **22**: 117–130.
- Bjerke L, Mackay A, Nandhabalan M, Burford A, Jury A, Popov S, Bax DA, Carvalho D, Taylor KR, Vinci M, et al. 2013. Histone H3.3 mutations drive pediatric glioblastoma through upregulation of MYCN. *Cancer Discov* **3**: 512–519.
- Blasina A, Hallin J, Chen E, Arango ME, Kraynov E, Register J, Grant S, Ninkovic S, Chen P, Nichols T, et al. 2008. Breaching the DNA damage checkpoint via PF-00477736, a novel small-molecule inhibitor of checkpoint kinase 1. *Mol Cancer Ther* **7**: 2394–2404.
- * Bradner J. 2013. Inhibiting Myc. *Cold Spring Harb Perspect Med* doi: 10.1101/cshperspect.014266.
- Breit S, Schwab M. 1989. Suppression of MYC by high expression of NMYC in human neuroblastoma cells. *J Neurosci Res* **24**: 21–28.
- Brenner C, Deplus R, Didelot C, Lorient A, Viré E, De Smet C, Gutierrez A, Danovi D, Bernard D, Boon T, et al. 2005. Myc represses transcription through recruitment of DNA methyltransferase corepressor. *EMBO J* **24**: 336–346.
- Brodeur GM, Seeger RC, Schwab M, Varmus HE, Bishop JM. 1984. Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. *Science* **224**: 1121–1124.
- Buechner J, Einvik C. 2012. N-myc and noncoding RNAs in neuroblastoma. *Mol Cancer Res* **10**: 1243–1253.
- Burkhardt CA, Cheng AJ, Madafoglio J, Kavallaris M, Mili M, Marshall GM, Weiss WA, Khachigian LM, Norris MD, Haber M. 2003. Effects of MYCN antisense oligonucleotide administration on tumorigenesis in a murine model of neuroblastoma. *J Natl Cancer Inst* **95**: 1394–1403.
- Capasso M, Devoto M, Hou C, Asgharzadeh S, Glessner JT, Attiyeh EF, Mossé YP, Kim C, Diskin SJ, Cole KA, et al. 2009. Common variations in *BARD1* influence susceptibility to high-risk neuroblastoma. *Nat Genet* **41**: 718–723.
- Capasso M, Diskin SJ, Totaro F, Longo L, Mariano MD, Russo R, Cimmino F, Hakonarson H, Tonini GB, Devoto M, et al. 2013. Replication of GWAS-identified neuroblastoma risk loci strengthens the role of *BARD1* and affirms the cumulative effect of genetic variations on disease susceptibility. *Carcinogenesis* **34**: 605–611.
- Carén H, Kryh H, Nethander M, Sjöberg R-M, Träger C, Nilsson S, Abrahamsson J, Kogner P, Martinsson T. 2010. High-risk neuroblastoma tumors with 11q-deletion display a poor prognostic, chromosome instability phenotype with later onset. *Proc Natl Acad Sci* **107**: 4323–4328.
- Caron H, Spieker N, Godfried M, Veenstra M, van Sluis P, de Kraker J, Voûte P, Versteeg R. 2001. Chromosome bands 1p35-36 contain two distinct neuroblastoma tumor suppressor loci, one of which is imprinted. *Genes Chromosomes Cancer* **30**: 168–174.
- Carpenter EL, Mossé YP. 2012. Targeting ALK in neuroblastoma—preclinical and clinical advancements. *Nat Rev Clin Oncol* **9**: 391–399.
- Casaccia-Bonnel P, Tikoo R, Kiyokawa H, Friedrich V, Chao MV, Koff A. 1997. Oligodendrocyte precursor differentiation is perturbed in the absence of the cyclin-dependent kinase inhibitor p27^{Kip1}. *Genes Dev* **11**: 2335–2346.
- Casciano I, Banelli B, Croce M, De Ambrosio A, Di Vinci A, Gelvi I, Pagnan G, Brignole C, Allemanni G, Ferrini S, et al. 2004. Caspase-8 gene expression in neuroblastoma. *Ann N Y Acad Sci* **1028**: 157–167.
- Cavalieri F, Goldfarb M. 1988. N-myc proto-oncogene expression can induce DNA replication in Balb/c 3T3 fibroblasts. *Oncogene* **2**: 289–291.
- Chanthery YH, Gustafson WC, Itsara M, Persson A, Hackett CS, Grimmer M, Charron E, Yakovenko S, Kim G, Matthay KK, et al. 2012. Paracrine signaling through MYCN enhances tumor-vascular interactions in neuroblastoma. *Sci Transl Med* **4**: 115ra3.
- * Chappell J, Dalton S. 2013. Roles for MYC in the establishment and maintenance of pluripotency. *Cold Spring Harb Perspect Med* doi: 10.1101/cshperspect.014381.
- Charron J, Malynn BA, Fisher P, Stewart V, Jeannotte L, Goff SP, Robertson EJ, Alt FW. 1992. Embryonic lethality in mice homozygous for a targeted disruption of the N-myc gene. *Genes Dev* **6**: 2248–2257.
- Chen L, Iraci N, Gherardi S, Gamble LD, Wood KM, Perini G, Lunec J, Tweddle DA. 2010. p53 is a direct transcriptional target of MYCN in neuroblastoma. *Cancer Res* **70**: 1377–1388.
- Chen Y, Takita J, Choi YL, Kato M, Ohira M, Sanada M, Wang L, Soda M, Kikuchi A, Igarashi T, et al. 2008. Oncogenic mutations of ALK kinase in neuroblastoma. *Nature* **455**: 971–974.
- Cheng NC, Van Roy N, Chan A, Beitsma M, Westerveld A, Speleman F, Versteeg R. 1995. Deletion mapping in neuroblastoma cell lines suggests two distinct tumor suppressor genes in the 1p35-36 region, only one of which is associated with N-myc amplification. *Oncogene* **10**: 291–297.
- Chesler L, Goldenberg DD, Collins R, Grimmer M, Kim GE, Tihan T, Nguyen K, Yakovenko S, Matthay KK, Weiss WA. 2008. Chemotherapy-induced apoptosis in a transgenic model of neuroblastoma proceeds through p53 induction. *Neoplasia* **10**: 1268–1274.
- Chesler L, Schlieve C, Goldenberg DD, Kenney A, Kim G, McMillan A, Matthay KK, Rowitch D, Weiss WA. 2006. Inhibition of phosphatidylinositol 3-kinase destabilizes Mycn protein and blocks malignant progression in neuroblastoma. *Cancer Res* **66**: 8139–8146.
- Cheung N-KV, Zhang J, Lu C, Parker M, Bahrami A, Tickoo SK, Heguy A, Pappo AS, Federico S, Dalton J, et al. 2012. Association of age at diagnosis and genetic mutations in patients with neuroblastoma. *JAMA* **307**: 1062–1071.
- Ciani E, Severi S, Contestabile A, Bartesaghi R, Contestabile A. 2004. Nitric oxide negatively regulates proliferation and promotes neuronal differentiation through N-Myc downregulation. *J Cell Sci* **117**: 4727–4737.
- Cinatl J, Cinatl J, Mainke M, Weissflog A, Rabenau H, Kornhuber B, Doerr HW. 1993. In vitro differentiation of human neuroblastoma cells induced by sodium phenylacetate. *Cancer Lett* **70**: 15–24.
- Clark O, Schmidt F, Coles CH, Tchetchelnitski V, Stoker AW. 2012. Functional analysis of the putative tumor suppressor



- por PTPRD in neuroblastoma cells. *Cancer Invest* **30**: 422–432.
- Cole KA, Huggins J, Laquaglia M, Hulderman CE, Russell MR, Bosse K, Diskin SJ, Attiyeh EF, Sennett R, Norris G, et al. 2011. RNAi screen of the protein kinome identifies checkpoint kinase 1 (CHK1) as a therapeutic target in neuroblastoma. *Proc Natl Acad Sci* **108**: 3336–3341.
- Corvetta D, Chayka O, Gherardi S, D'Acuneto CW, Cantilena S, Valli E, Piotrowska I, Perini G, Sala A. 2013. Physical interaction between MYCN oncogene and polycomb repressive complex 2 (PRC2) in neuroblastoma: Functional and therapeutic implications. *J Biol Chem* **288**: 8332–8341.
- Cotterman R, Knoepfler PS. 2009. N-Myc regulates expression of pluripotency genes in neuroblastoma including *lif*, *klf2*, *klf4*, and *lin28b*. *PLoS One* **4**: e5799.
- Davis AC, Wims M, Spotts GD, Hann SR, Bradley A. 1993. A null *c-myc* mutation causes lethality before 10.5 days of gestation in homozygotes and reduced fertility in heterozygous female mice. *Genes Dev* **7**: 671–682.
- De Brouwer S, De Preter K, Kumps C, Zabrocki P, Porcu M, Westerhout EM, Lakeman A, Vandenspele J, Hoebeek J, Van Maerken T, et al. 2010. Meta-analysis of neuroblastomas reveals a skewed *ALK* mutation spectrum in tumors with MYCN amplification. *Clin Cancer Res* **16**: 4353–4362.
- De La Fuente R, Baumann C, Viveiros MM. 2011. Role of ATRX in chromatin structure and function: Implications for chromosome instability and human disease. *Reproduction* **142**: 221–234.
- Delmore JE, Issa GC, Lemieux ME, Rahl PB, Shi J, Jacobs HM, Kastiris E, Gilpatrick T, Paranal RM, Qi J, et al. 2011. BET bromodomain inhibition as a therapeutic strategy to target *c-Myc*. *Cell* **146**: 904–917.
- Dewitt J, Ochoa V, Urschitz J, Elston M, Moisyadi S, Nishi R. 2013. Constitutively active TrkB confers an aggressive transformed phenotype to a neural crest-derived cell line. *Oncogene*. doi: 10.1038/onc.2013.39.
- Diskin SJ, Capasso M, Schnepf RW, Cole KA, Attiyeh EF, Hou C, Diamond M, Carpenter EL, Winter C, Lee H, et al. 2012. Common variation at 6q16 within *HACE1* and *LIN28B* influences susceptibility to neuroblastoma. *Nat Genet* **44**: 1126–1130.
- DuBois SG, Kalika Y, Lukens JN, Brodeur GM, Seeger RC, Atkinson JB, Haase GM, Black CT, Perez C, Shimada H, et al. 1999. Metastatic sites in stage IV and IVS neuroblastoma correlate with age, tumor biology, and survival. *J Pediatr Hematol Oncol* **21**: 181–189.
- Dungwa JV, Uparkar U, May MT, Ramani P. 2012. Angiogenesis up-regulation correlates with adverse clinicopathological and biological factors, increased microvascular density and poor patient outcome in neuroblastomas. *Histopathology* **60**: 911–923.
- Eggert A, Grotzer MA, Zuzak TJ, Wiewrodt BR, Ho R, Ikegaki N, Brodeur GM. 2001. Resistance to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis in neuroblastoma cells correlates with a loss of caspase-8 expression. *Cancer Res* **61**: 1314–1319.
- Elson A, Deng C, Campos-Torres J, Donehower LA, Leder P. 1995. The MMTV/*c-myc* transgene and p53 null alleles collaborate to induce T-cell lymphomas, but not mammary carcinomas in transgenic mice. *Oncogene* **11**: 181–190.
- Evan GI, Wyllie AH, Gilbert CS, Littlewood TD, Land H, Brooks M, Waters CM, Penn LZ, Hancock DC. 1992. Induction of apoptosis in fibroblasts by *c-myc* protein. *Cell* **69**: 119–128.
- Faisal A, Vaughan L, Bavetsias V, Sun C, Atrash B, Avery S, Jamin Y, Robinson SP, Workman P, Blagg J, et al. 2011. The aurora kinase inhibitor CCT137690 downregulates MYCN and sensitizes MYCN-amplified neuroblastoma in vivo. *Mol Cancer Ther* **10**: 2115–2123.
- * Farrell AS, Sears RC. 2014. MYC degradation. *Cold Spring Harb Perspect Med*. doi: 10.1101/cshperspect.a014365.
- Fotsis T, Breit S, Lutz W, Rössler J, Hatzl E, Schwab M, Schweigerer L. 1999. Down-regulation of endothelial cell growth inhibitors by enhanced MYCN oncogene expression in human neuroblastoma cells. *Eur J Biochem* **263**: 757–764.
- Fulda S, Lutz W, Schwab M, Debatin KM. 2000. MycN sensitizes neuroblastoma cells for drug-triggered apoptosis. *Med Pediatr Oncol* **35**: 582–584.
- Fulda S, Poremba C, Berwanger B, Häcker S, Eilers M, Christiansen H, Hero B, Debatin K-M. 2006. Loss of caspase-8 expression does not correlate with MYCN amplification, aggressive disease, or prognosis in neuroblastoma. *Cancer Res* **66**: 10016–10023.
- Gamble LD, Kees UR, Tweddle DA, Lunec J. 2012. MYCN sensitizes neuroblastoma to the MDM2-p53 antagonists Nutlin-3 and MI-63. *Oncogene* **31**: 752–763.
- George RE, Sanda T, Hanna M, Fröhling S, Luther W, Zhang J, Ahn Y, Zhou W, London WB, McGrady P, et al. 2008. Activating mutations in *ALK* provide a therapeutic target in neuroblastoma. *Nature* **455**: 975–978.
- Gogolin S, Ehemann V, Becker G, Brueckner LM, Dreidax D, Bannert S, Nolte I, Savelyeva L, Bell E, Westermann F. 2013. CDK4 inhibition restores G₁-S arrest in MYCN-amplified neuroblastoma cells in the context of doxorubicin-induced DNA damage. *Cell Cycle* **12**: 1091–1104.
- Golubovskaya VM, Finch R, Kweh F, Massoll NA, Campbell-Thompson M, Wallace MR, Cance WG. 2008. p53 regulates FAK expression in human tumor cells. *Mol Carcinog* **47**: 373–382.
- Gómez-Casares MT, García-Alegria E, López-Jorge CE, Ferrándiz N, Blanco R, Alvarez S, Vaqué JP, Bretones G, Caraballo JM, Sánchez-Bailón P, et al. 2013. MYC antagonizes the differentiation induced by imatinib in chronic myeloid leukemia cells through downregulation of p27(KIP1). *Oncogene* **32**: 2239–2246.
- Gonzalez-Gomez P, Bello MJ, Lomas J, Arjona D, Alonso ME, Amiñoso C, Lopez-Marin I, Anselmo NP, Sarasa JL, Gutierrez M, et al. 2003. Aberrant methylation of multiple genes in neuroblastic tumours. Relationship with MYCN amplification and allelic status at 1p. *Eur J Cancer* **39**: 1478–1485.
- Goodman LA, Liu BC, Thiele CJ, Schmidt ML, Cohn SL, Yamashiro JM, Pai DS, Ikegaki N, Wada RK. 1997. Modulation of N-myc expression alters the invasiveness of neuroblastoma. *Clin Exp Metastasis* **15**: 130–139.
- Gu L, Zhang H, He J, Li J, Huang M, Zhou M. 2012. MDM2 regulates MYCN mRNA stabilization and translation in human neuroblastoma cells. *Oncogene* **31**: 1342–1353.

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- Gualdrini F, Corvetta D, Cantilena S, Chayka O, Tanno B, Raschella G, Sala A. 2010. Addiction of MYCN amplified tumours to B-MYB underscores a reciprocal regulatory loop. *Oncotarget* **1**: 278–288.
- Gustafson WC, Weiss WA. 2010. Myc proteins as therapeutic targets. *Oncogene* **29**: 1249–1259.
- Han S, Wada RK, Sidell N. 2001. Differentiation of human neuroblastoma by phenylacetate is mediated by peroxisome proliferator-activated receptor γ . *Cancer Res* **61**: 3998–4002.
- Hartl M, Mitterstiller A-M, Valovka T, Breuker K, Hobmayer B, Bister K. 2010. Stem cell-specific activation of an ancestral myc protooncogene with conserved basic functions in the early metazoan Hydra. *Proc Natl Acad Sci* **107**: 4051–4056.
- Hatton BA, Knoepfler PS, Kenney AM, Rowitch DH, de Alborán IM, Olson JM, Eisenman RN. 2006. N-myc is an essential downstream effector of Shh signaling during both normal and neoplastic cerebellar growth. *Cancer Res* **66**: 8655–8661.
- Hatzi E, Breit S, Zoepfel A, Ashman K, Tontsch U, Ahorn H, Murphy C, Schweigerer L, Fotsis T. 2000. MYCN oncogene and angiogenesis: Down-regulation of endothelial growth inhibitors in human neuroblastoma cells. Purification, structural, and functional characterization. *Adv Exp Med Biol* **476**: 239–248.
- Hatzi E, Murphy C, Zoepfel A, Ahorn H, Tontsch U, Bamberg A-M, Yamauchi-Takahara K, Schweigerer L, Fotsis T. 2002a. N-myc oncogene overexpression down-regulates leukemia inhibitory factor in neuroblastoma. *Eur J Biochem* **269**: 3732–3741.
- Hatzi E, Murphy C, Zoepfel A, Rasmussen H, Morbidelli L, Ahorn H, Kunisada K, Tontsch U, Klenk M, Yamauchi-Takahara K, et al. 2002b. N-myc oncogene overexpression down-regulates IL-6; evidence that IL-6 inhibits angiogenesis and suppresses neuroblastoma tumor growth. *Oncogene* **21**: 3552–3561.
- Helland Å, Anglesio MS, George J, Cowin PA, Johnstone CN, House CM, Sheppard KE, Etamadmoghadam D, Melnyk N, Rustgi AK, et al. 2011. Deregulation of MYCN, LIN28B and LET7 in a molecular subtype of aggressive high-grade serous ovarian cancers. *PLoS One* **6**: e18064.
- Henriksen JR, Haug BH, Buechner J, Tømte E, Løkke C, Flægstad T, Einvik C. 2011. Conditional expression of retrovirally delivered anti-MYCN shRNA as an in vitro model system to study neuronal differentiation in MYCN-amplified neuroblastoma. *BMC Dev Biol* **11**: 1.
- Herbst A, Hemann MT, Tworkowski KA, Salghetti SE, Lowe SW, Tansey WP. 2005. A conserved element in Myc that negatively regulates its proapoptotic activity. *EMBO Rep* **6**: 177–183.
- Heukamp LC, Thor T, Schramm A, De Preter K, Kumps C, De Wilde B, Odersky A, Peifer M, Lindner S, Spruessel A, et al. 2012. Targeted expression of mutated ALK induces neuroblastoma in transgenic mice. *Sci Transl Med* **4**: 141ra91.
- Hiyama E, Hiyama K, Ohtsu K, Yamaoka H, Fukuba I, Matsuura Y, Yokoyama T. 2001. Biological characteristics of neuroblastoma with partial deletion in the short arm of chromosome 1. *Med Pediatr Oncol* **36**: 67–74.
- Ho R, Eggert A, Hishiki T, Minturn JE, Ikegaki N, Foster P, Camoratto AM, Evans AE, Brodeur GM. 2002. Resistance to chemotherapy mediated by TrkB in neuroblastomas. *Cancer Res* **62**: 6462–6466.
- Hossain MS, Ozaki T, Wang H, Nakagawa A, Takenobu H, Ohira M, Kamijo T, Nakagawara A. 2008. N-MYC promotes cell proliferation through a direct transactivation of neuronal leucine-rich repeat protein-1 (NLRR1) gene in neuroblastoma. *Oncogene* **27**: 6075–6082.
- Hossain S, Takatori A, Nakamura Y, Suenaga Y, Kamijo T, Nakagawara A. 2012. NLRR1 enhances EGF-mediated MYCN induction in neuroblastoma and accelerates tumor growth in vivo. *Cancer Res* **72**: 4587–4596.
- Huang R, Cheung N-KV, Vider J, Cheung IY, Gerald WL, Tickoo SK, Holland EC, Blasberg RG. 2011. MYCN and MYC regulate tumor proliferation and tumorigenesis directly through BMI1 in human neuroblastomas. *FASEB J* **25**: 4138–4149.
- Iavarone A, Garg P, Lasorella A, Hsu J, Israel MA. 1994. The helix-loop-helix protein Id-2 enhances cell proliferation and binds to the retinoblastoma protein. *Genes Dev* **8**: 1270–1284.
- Iolascon A, Borriello A, Giordani L, Cucciolla V, Moretti A, Monno F, Criniti V, Marzullo A, Criscuolo M, Ragione FD. 2003. Caspase 3 and 8 deficiency in human neuroblastoma. *Cancer Genet Cytogenet* **146**: 41–47.
- Iraci N, Diolaiti D, Papa A, Porro A, Valli E, Gherardi S, Herold S, Eilers M, Bernardoni R, Valle Della G, et al. 2011. A SP1/MIZ1/MYCN repression complex recruits HDAC1 at the TRKA and p75NTR promoters and affects neuroblastoma malignancy by inhibiting the cell response to NGE. *Cancer Res* **71**: 404–412.
- Izumi H, Kaneko Y. 2012. Evidence of asymmetric cell division and centrosome inheritance in human neuroblastoma cells. *Proc Natl Acad Sci* **109**: 18048–18053.
- Janoueix-Lerosey I, Lequin D, Brugières L, Ribeiro A, de Pontual L, Combaret V, Raynal V, Puisieux A, Schleiermacher G, Pierron G, et al. 2008. Somatic and germline activating mutations of the ALK kinase receptor in neuroblastoma. *Nature* **455**: 967–970.
- Johnsen JI, Segerström L, Orrego A, Elfman L, Henriksson M, Kågedal B, Eksborg S, Sveinbjörnsson B, Kogner P. 2008. Inhibitors of mammalian target of rapamycin downregulate MYCN protein expression and inhibit neuroblastoma growth in vitro and in vivo. *Oncogene* **27**: 2910–2922.
- Kang J, Rychahou PG, Ishola TA, Mourot JM, Evers BM, Chung DH. 2008. N-myc is a novel regulator of PI3K-mediated VEGF expression in neuroblastoma. *Oncogene* **27**: 3999–4007.
- Kang J-H, Rychahou PG, Ishola TA, Qiao J, Evers BM, Chung DH. 2006. MYCN silencing induces differentiation and apoptosis in human neuroblastoma cells. *Biochem Biophys Res Commun* **351**: 192–197.
- Kawauchi D, Robinson G, Uziel T, Gibson P, Reh J, Gao C, Finkelstein D, Qu C, Pounds S, Ellison DW, et al. 2012. A mouse model of the most aggressive subgroup of human medulloblastoma. *Cancer Cell* **21**: 168–180.
- Kenney AM, Cole MD, Rowitch DH. 2003. Nmyc upregulation by sonic hedgehog signaling promotes proliferation in developing cerebellar granule neuron precursors. *Development* **130**: 15–28.



- Killela PJ, Reitman ZJ, Jiao Y, Bettegowda C, Agrawal N, Diaz LA, Friedman AH, Friedman H, Gallia GL, Giovannella BC, et al. 2013. TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. *Proc Natl Acad Sci* **110**: 6021–6026.
- Kim SS, Shago M, Kaustov L, Boutros PC, Clendening JW, Sheng Y, Trentin GA, Barsyte-Lovejoy D, Mao DYL, Kay R, et al. 2007. *CUL7* is a novel antiapoptotic oncogene. *Cancer Res* **67**: 9616–9622.
- Knoepfler PS, Cheng PF, Eisenman RN. 2002. *N-myc* is essential during neurogenesis for the rapid expansion of progenitor cell populations and the inhibition of neuronal differentiation. *Genes Dev* **16**: 2699–2712.
- Knoepfler PS, Zhang X-Y, Cheng PF, Gafken PR, McMahon SB, Eisenman RN. 2006. Myc influences global chromatin structure. *EMBO J* **25**: 2723–2734.
- Kohl NE, Kanda N, Schreck RR, Bruns G, Latt SA, Gilbert F, Alt FW. 1983. Transposition and amplification of oncogene-related sequences in human neuroblastomas. *Cell* **35**: 359–367.
- Kohl NE, Legouy E, DePinho RA, Nisen PD, Smith RK, Gee CE, Alt FW. 1986. Human *N-myc* is closely related in organization and nucleotide sequence to *c-myc*. *Nature* **319**: 73–77.
- Komuro H, Valentine MB, Rowe ST, Kidd VJ, Makino S, Brodeur GM, Cohn SL, Look AT. 1998. Fluorescence in situ hybridization analysis of chromosome 1p36 deletions in human MYCN amplified neuroblastoma. *J Pediatr Surg* **33**: 1695–1698.
- Koppen A, Ait-Aissa R, Hopman S, Koster J, Haneveld E, Versteeg R, Valentijn LJ. 2007a. Dickkopf-1 is down-regulated by MYCN and inhibits neuroblastoma cell proliferation. *Cancer Lett* **256**: 218–228.
- Koppen A, Ait-Aissa R, Koster J, van Sluis PG, Ora I, Caron HN, Volckmann R, Versteeg R, Valentijn LJ. 2007b. Direct regulation of the minichromosome maintenance complex by MYCN in neuroblastoma. *Eur J Cancer* **43**: 2413–2422.
- Kotake Y, Cao R, Viatour P, Sage J, Zhang Y, Xiong Y. 2007. pRB family proteins are required for H3K27 trimethylation and Polycomb repression complexes binding to and silencing p16^{INK4a} tumor suppressor gene. *Genes Dev* **21**: 49–54.
- Lasorella A, Boldrini R, Dominici C, Donfrancesco A, Yokota Y, Inserra A, Iavarone A. 2002. Id2 is critical for cellular proliferation and is the oncogenic effector of *N-myc* in human neuroblastoma. *Cancer Res* **62**: 301–306.
- Lasorella A, Iavarone A, Israel MA. 1996. Id2 specifically alters regulation of the cell cycle by tumor suppressor proteins. *Mol Cell Biol* **16**: 2570–2578.
- Lasorella A, Noseda M, Beyna M, Yokota Y, Iavarone A. 2000. Id2 is a retinoblastoma protein target and mediates signalling by Myc oncoproteins. *Nature* **407**: 592–598.
- Laurenti E, Varnum-Finney B, Wilson A, Ferrero I, Blanco-Bose WE, Ehninger A, Knoepfler PS, Cheng PF, MacDonald HR, Eisenman RN, et al. 2008. Hematopoietic stem cell function and survival depend on *c-Myc* and *N-Myc* activity. *Cell Stem Cell* **3**: 611–624.
- Lázcoz P, Muñoz J, Nistal M, Pestaña A, Encío I, Castresana JS. 2006. Frequent promoter hypermethylation of RASSF1A and CASP8 in neuroblastoma. *BMC Cancer* **6**: 254.
- Liu T, Tee AEL, Porro A, Smith SA, Dwarte T, Liu PY, Iraci N, Sekyere E, Haber M, Norris MD, et al. 2007. Activation of tissue transglutaminase transcription by histone deacetylase inhibition as a therapeutic approach for Myc oncogenesis. *Proc Natl Acad Sci* **104**: 18682–18687.
- Lovén J, Zinin N, Wahlström T, Müller I, Brodin P, Fredlund E, Ribacke U, Pivarcsi A, Pahlman S, Henriksson M. 2010. MYCN-regulated microRNAs repress estrogen receptor- α (*ESR1*) expression and neuronal differentiation in human neuroblastoma. *Proc Natl Acad Sci* **107**: 1553–1558.
- Lutterbach B, Hann SR. 1994. Hierarchical phosphorylation at N-terminal transformation-sensitive sites in *c-Myc* protein is regulated by mitogens and in mitosis. *Mol Cell Biol* **14**: 5510–5522.
- Ma L, Young J, Prabhala H, Pan E, Mestdagh P, Muth D, Teruya-Feldstein J, Reinhardt F, Onder TT, Valastyan S, et al. 2010. miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. *Nat Cell Biol* **12**: 247–256.
- Malynn BA, de Alboran IM, O'Hagan RC, Bronson R, Davidson L, DePinho RA, Alt FW. 2000. *N-myc* can functionally replace *c-myc* in murine development, cellular growth, and differentiation. *Genes Dev* **14**: 1390–1399.
- Maris JM, Hogarty MD, Bagatell R, Cohn SL. 2007. Neuroblastoma. *Lancet* **369**: 2106–2120.
- Marshall GM, Gherardi S, Xu N, Neiron Z, Trahair T, Scarlett CJ, Chang DK, Liu PY, Jankowski K, Iraci N, et al. 2010. Transcriptional upregulation of histone deacetylase 2 promotes Myc-induced oncogenic effects. *Oncogene* **29**: 5957–5968.
- Marshall GM, Liu PY, Gherardi S, Scarlett CJ, Bedalov A, Xu N, Iraci N, Valli E, Ling D, Thomas W, et al. 2011. SIRT1 promotes *N-Myc* oncogenesis through a positive feedback loop involving the effects of MKP3 and ERK on *N-Myc* protein stability. *PLoS Genet* **7**: e1002135.
- Matsumoto M, Akiyama T, Miyatake S, Oda Y, Kikuchi H, Hanaoka M, Namba Y. 1989. Expression of proto-oncogene products during drug-induced differentiation of a neuroblastoma cell line SK-N-DZ. *Acta Neuropathol* **79**: 217–221.
- Matthay KK, George RE, Yu AL. 2012. Promising therapeutic targets in neuroblastoma. *Clin Cancer Res* **18**: 2740–2753.
- Meehan M, Parthasarathi L, Moran N, Jefferies CA, Foley N, Lazzari E, Murphy D, Ryan J, Ortiz B, Fabius AWM, et al. 2012. Protein tyrosine phosphatase receptor delta acts as a neuroblastoma tumor suppressor by destabilizing the aurora kinase A oncogene. *Mol Cancer* **11**: 6.
- Megison ML, Stewart JE, Nabers HC, Gillory LA, Beierle EA. 2012. FAK inhibition decreases cell invasion, migration and metastasis in MYCN amplified neuroblastoma. *Clin Exp Metastasis* **18**: 2740–2753.
- Meitar D, Crawford SE, Rademaker AW, Cohn SL. 1996. Tumor angiogenesis correlates with metastatic disease, *N-myc* amplification, and poor outcome in human neuroblastoma. *J Clin Oncol* **14**: 405–414.
- Meyer N, Penn LZ. 2008. Reflecting on 25 years with MYC. *Nat Rev Cancer* **8**: 976–990.

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- Molenaar JJ, Domingo-Fernández R, Ebus ME, Lindner S, Koster J, Drabek K, Mestdagh P, van Sluis P, Valentijn LJ, van Nes J, et al. 2012a. LIN28B induces neuroblastoma and enhances MYCN levels via let-7 suppression. *Nat Genet* **44**: 1199–1206.
- Molenaar JJ, Koster J, Zwijnenburg DA, van Sluis P, Valentijn LJ, van der Ploeg I, Hamdi M, van Nes J, Westerman BA, van Arkel J, et al. 2012b. Sequencing of neuroblastoma identifies chromothripsis and defects in neurogenesis genes. *Nature* **483**: 589–593.
- Mossé YP, Diskin SJ, Wasserman N, Rinaldi K, Attiyeh EF, Cole K, Jagannathan J, Bhambhani K, Winter C, Maris JM. 2007. Neuroblastomas have distinct genomic DNA profiles that predict clinical phenotype and regional gene expression. *Genes Chromosomes Cancer* **46**: 936–949.
- Mossé YP, Laudenslager M, Longo L, Cole KA, Wood A, Attiyeh EF, Laquaglia MJ, Sennett R, Lynch JE, Perri P, et al. 2008. Identification of ALK as a major familial neuroblastoma predisposition gene. *Nature* **455**: 930–935.
- Mueller S, Matthay KK. 2009. Neuroblastoma: Biology and staging. *Curr Oncol Rep* **11**: 431–438.
- Muth D, Ghazaryan S, Eckerle I, Beckett E, Pöhler C, Batzler J, Beisel C, Gogolin S, Fischer M, Henrich K-O, et al. 2010. Transcriptional repression of SKP2 is impaired in MYCN-amplified neuroblastoma. *Cancer Res* **70**: 3791–3802.
- Nakagawa M, Takizawa N, Narita M, Ichisaka T, Yamanaka S. 2010. Promotion of direct reprogramming by transformation-deficient Myc. *Proc Natl Acad Sci* **107**: 14152–14157.
- Nakagawara A, Azar CG, Scavarda NJ, Brodeur GM. 1994. Expression and function of TRK-B and BDNF in human neuroblastomas. *Mol Cell Biol* **14**: 759–767.
- Nara K, Kusafuka T, Yoneda A, Oue T, Sangkhathat S, Fukuzawa M. 2007. Silencing of MYCN by RNA interference induces growth inhibition, apoptotic activity and cell differentiation in a neuroblastoma cell line with MYCN amplification. *Int J Oncol* **30**: 1189–1196.
- Nguyen LB, Diskin SJ, Capasso M, Wang K, Diamond MA, Glessner J, Kim C, Attiyeh EF, Mossé YP, Cole K, et al. 2011. Phenotype restricted genome-wide association study using a gene-centric approach identifies three low-risk neuroblastoma susceptibility Loci. *PLoS Genet* **7**: e1002026.
- Noujaim D, van Golen CM, van Golen KL, Grauman A, Feldman EL. 2002. N-Myc and Bcl-2 coexpression induces MMP-2 secretion and activation in human neuroblastoma cells. *Oncogene* **21**: 4549–4557.
- Ochiai H, Takenobu H, Nakagawa A, Yamaguchi Y, Kimura M, Ohira M, Okimoto Y, Fujimura Y, Koseki H, Kohno Y, et al. 2010. Bmi1 is a MYCN target gene that regulates tumorigenesis through repression of *KIF1B* and *TSLC1* in neuroblastoma. *Oncogene* **29**: 2681–2690.
- Otto T, Horn S, Brockmann M, Eilers U, Schüttrumpf L, Popov N, Kenney AM, Schulte JH, Beijersbergen R, Christiansen H, et al. 2009. Stabilization of N-Myc is a critical function of Aurora A in human neuroblastoma. *Cancer Cell* **15**: 67–78.
- Ozer E, Altungoz O, Unlu M, Aygun N, Tumer S, Olgun N. 2007. Association of MYCN amplification and 1p deletion in neuroblastomas with high tumor vascularity. *Appl Immunohistochem Mol Morphol* **15**: 181–186.
- Pei Y, Moore CE, Wang J, Tewari AK, Eroshkin A, Cho Y-J, Witt H, Korshunov A, Read T-A, Sun JL, et al. 2012. An animal model of MYC-driven medulloblastoma. *Cancer Cell* **21**: 155–167.
- Plantaz D, Mohapatra G, Matthay KK, Pellarin M, Seeger RC, Feuerstein BG. 1997. Gain of chromosome 17 is the most frequent abnormality detected in neuroblastoma by comparative genomic hybridization. *Am J Pathol* **150**: 81–89.
- Pugh TJ, Morozova O, Attiyeh EF, Asgharzadeh S, Wei JS, Auclair D, Carter SL, Cibulskis K, Hanna M, Kiezun A, et al. 2013. The genetic landscape of high-risk neuroblastoma. *Nat Genet* **45**: 279–284.
- Pugh TJ, Weeraratne SD, Archer TC, Pomeranz Krummel DA, Auclair D, Bochicchio J, Carneiro MO, Carter SL, Cibulskis K, Erlich RL, et al. 2012. Medulloblastoma exome sequencing uncovers subtype-specific somatic mutations. *Nature* **488**: 106–110.
- Puissant A, Frumm SM, Alexe G, Bassil CF, Qi J, Chanthery YH, Nekritz EA, Zeid R, Gustafson WC, Greninger P, et al. 2013. Targeting MYCN in neuroblastoma by BET bromodomain inhibition. *Cancer Discov* **3**: 308–323.
- Pulverer BJ, Fisher C, Vousden K, Littlewood T, Evan G, Woodgett JR. 1994. Site-specific modulation of c-Myc cotransformation by residues phosphorylated in vivo. *Oncogene* **9**: 59–70.
- Raschella G, Cesi V, Amendola R, Negroni A, Tanno B, Altavista P, Tonini GP, De Bernardi B, Calabretta B. 1999. Expression of B-myc in neuroblastoma tumors is a poor prognostic factor independent from MYCN amplification. *Cancer Res* **59**: 3365–3368.
- Reddy CD, Patti R, Guttapalli A, Maris JM, Yanamandra N, Rachamalla A, Sutton LN, Phillips PC, Posner GH. 2006. Anticancer effects of the novel 1 α , 25-dihydroxyvitamin D3 hybrid analog QW1624F2-2 in human neuroblastoma. *J Cell Biochem* **97**: 198–206.
- Ribatti D, Raffaghello L, Pastorino F, Nico B, Brignole C, Vacca A, Ponzoni M. 2002. In vivo angiogenic activity of neuroblastoma correlates with MYCN oncogene overexpression. *Int J Cancer* **102**: 351–354.
- Rosenbaum H, Webb E, Adams JM, Cory S, Harris AW. 1989. N-myc transgene promotes B lymphoid proliferation, elicits lymphomas and reveals cross-regulation with c-myc. *EMBO J* **8**: 749–755.
- * Roussel MF, Robinson GW. 2013. Role of MYC in medulloblastoma. *Cold Spring Harb Perspect Med* doi: 10.1101/cshperspect.014308.
- * Sabó A, Amati B. 2014. Genome recognition by Myc. *Cold Spring Harb Perspect Med* doi: 10.1101/cshperspect.014191.
- Sausen M, Leary RJ, Jones S, Wu J, Reynolds CP, Liu X, Blackford A, Parmigiani G, Diaz LA, Papadopoulos N, et al. 2013. Integrated genomic analyses identify *ARID1A* and *ARID1B* alterations in the childhood cancer neuroblastoma. *Nat Genet* **45**: 12–17.
- Sawai S, Shimono A, Wakamatsu Y, Palmes C, Hanaoka K, Kondoh H. 1993. Defects of embryonic organogenesis resulting from targeted disruption of the N-myc gene in the mouse. *Development* **117**: 1445–1455.



- Schönherr C, Ruuth K, Kamaraj S, Wang C-L, Yang H-L, Combaret V, Djos A, Martinsson T, Christensen JG, Palmer RH, et al. 2012. Anaplastic Lymphoma Kinase (ALK) regulates initiation of transcription of MYCN in neuroblastoma cells. *Oncogene* **31**: 5193–5200.
- Schulte JH, Lindner S, Bohrer A, Maurer J, De Preter K, Lefever S, Heukamp L, Schulte S, Molenaar J, Versteeg R, et al. 2012. MYCN and ALKF1174L are sufficient to drive neuroblastoma development from neural crest progenitor cells. *Oncogene* **32**: 1059–1065.
- Schwab M, Alitalo K, Klempnauer KH, Varmus HE, Bishop JM, Gilbert F, Brodeur G, Goldstein M, Trent J. 1983. Amplified DNA with limited homology to *myc* cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumour. *Nature* **305**: 245–248.
- Schwab M, Varmus HE, Bishop JM. 1985. Human N-myc gene contributes to neoplastic transformation of mammalian cells in culture. *Nature* **316**: 160–162.
- Schwartztruber J, Korshunov A, Liu X-Y, Jones DTW, Pfaff E, Jacob K, Sturm D, Fontebasso AM, Quang D-AK, Tonjes M, et al. 2012. Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature* **482**: 226–231.
- Sears R, Nuckolls F, Haura E, Taya Y, Tamai K, Nevins JR. 2000. Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. *Genes Dev* **14**: 2501–2514.
- Seeger RC, Brodeur GM, Sather H, Dalton A, Siegel SE, Wong KY, Hammond D. 1985. Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. *N Engl J Med* **313**: 1111–1116.
- Seth A, Alvarez E, Gupta S, Davis RJ. 1991. A phosphorylation site located in the NH₂-terminal domain of c-Myc increases transactivation of gene expression. *J Biol Chem* **266**: 23521–23524.
- Shang X, Burlingame SM, Okcu MF, Ge N, Russell HV, Egler RA, David RD, Vasudevan SA, Yang J, Nuchtern JG. 2009. Aurora A is a negative prognostic factor and a new therapeutic target in human neuroblastoma. *Mol Cancer Ther* **8**: 2461–2469.
- Shioda N, Beppu H, Fukuda T, Li E, Kitajima I, Fukunaga K. 2011. Aberrant calcium/calmodulin-dependent protein kinase II (CaMKII) activity is associated with abnormal dendritic spine morphology in the *ATRX* mutant mouse brain. *J Neurosci* **31**: 346–358.
- Sidell N. 1982. Retinoic acid-induced growth inhibition and morphologic differentiation of human neuroblastoma cells in vitro. *J Natl Cancer Inst* **68**: 589–596.
- Sjostrom SK, Finn G, Hahn WC, Rowitch DH, Kenney AM. 2005. The Cdk1 complex plays a prime role in regulating N-myc phosphorylation and turnover in neural precursors. *Dev Cell* **9**: 327–338.
- Slack A, Chen Z, Tonelli R, Pule M, Hunt L, Pession A, Shohet JM. 2005. The p53 regulatory gene *MDM2* is a direct transcriptional target of MYCN in neuroblastoma. *Proc Natl Acad Sci* **102**: 731–736.
- Slavc I, Ellenbogen R, Jung WH, Vawter GF, Kretschmar C, Grier H, Korf BR. 1990. *myc* Gene amplification and expression in primary human neuroblastoma. *Cancer Res* **50**: 1459–1463.
- Song L, Ara T, Wu H-W, Woo C-W, Reynolds CP, Seeger RC, DeClerck YA, Thiele CJ, Sposto R, Metelitsa LS. 2007. Oncogene MYCN regulates localization of NKT cells to the site of disease in neuroblastoma. *J Clin Invest* **117**: 2702–2712.
- Stanton LW, Schwab M, Bishop JM. 1986. Nucleotide sequence of the human N-myc gene. *Proc Natl Acad Sci* **83**: 1772–1776.
- Stanton BR, Perkins AS, Tessarollo L, Sassoon DA, Parada LF. 1992. Loss of N-myc function results in embryonic lethality and failure of the epithelial component of the embryo to develop. *Genes Dev* **6**: 2235–2247.
- Strasser A, Harris AW, Bath ML, Cory S. 1990. Novel primitive lymphoid tumours induced in transgenic mice by cooperation between *myc* and *bcl-2*. *Nature* **348**: 331–333.
- Stupack DG, Puente XS, Boutsaboualoy S, Storgard CM, Cheresch DA. 2001. Apoptosis of adherent cells by recruitment of caspase-8 to unligated integrins. *J Cell Biol* **155**: 459–470.
- Stupack DG, Teitz T, Potter MD, Mikolon D, Houghton PJ, Kidd VJ, Lahti JM, Cheresch DA. 2006. Potentiation of neuroblastoma metastasis by loss of caspase-8. *Nature* **439**: 95–99.
- Swarbrick A, Woods SL, Shaw A, Balakrishnan A, Phua Y, Nguyen A, Chanthery Y, Lim L, Ashton LJ, Judson RL, et al. 2010. miR-380-5p represses p53 to control cellular survival and is associated with poor outcome in MYCN-amplified neuroblastoma. *Nat Med* **16**: 1134–1140.
- Swartling FJ, Savov V, Persson AI, Chen J, Hackett CS, Northcott PA, Grimmer MR, Lau J, Chesler L, Perry A, et al. 2012. Distinct neural stem cell populations give rise to disparate brain tumors in response to N-MYC. *Cancer Cell* **21**: 601–613.
- Tanaka N, Fukuzawa M. 2008. MYCN downregulates integrin $\alpha 1$ to promote invasion of human neuroblastoma cells. *Int J Oncol* **33**: 815–821.
- Teitz T, Inoue M, Valentine MB, Zhu K, Reh JE, Zhao W, Finkelstein D, Wang Y-D, Johnson MD, Calabrese C, et al. 2013. Th-MYCN mice with caspase-8 deficiency develop advanced neuroblastoma with bone marrow metastasis. *Cancer Res* **73**: 4086–4097.
- Teitz T, Wei T, Valentine MB, Vanin EE, Grenet J, Valentine VA, Behm FG, Look AT, Lahti JM, Kidd VJ. 2000. Caspase 8 is deleted or silenced preferentially in childhood neuroblastomas with amplification of MYCN. *Nat Med* **6**: 529–535.
- Thiele CJ, Reynolds CP, Israel MA. 1985. Decreased expression of N-myc precedes retinoic acid-induced morphological differentiation of human neuroblastoma. *Nature* **313**: 404–406.
- Tweddle DA, Malcolm AJ, Cole M, Pearson AD, Lunec J. 2001. p53 cellular localization and function in neuroblastoma: Evidence for defective G₁ arrest despite WAF1 induction in MYCN-amplified cells. *Am J Pathol* **158**: 2067–2077.
- Valentijn LJ, Koppen A, van Asperen R, Root HA, Haneveld F, Versteeg R. 2005. Inhibition of a new differentiation pathway in neuroblastoma by copy number defects of N-myc, *Cdc42*, and *nm23* genes. *Cancer Res* **65**: 3136–3145.
- Valli E, Trazzi S, Fuchs C, Erriquez D, Bartsaghi R, Perini G, Ciani E. 2012. CDKL5, a novel MYCN-repressed gene, blocks cell cycle and promotes differentiation of neuronal cells. *Biochim Biophys Acta* **1819**: 1173–1185.

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- Valsesia-Wittmann S, Magdeleine M, Dupasquier S, Garin E, Jallas A-C, Combaret V, Krause A, Leissner P, Puisieux A. 2004. Oncogenic cooperation between H-Twist and N-Myc overrides failsafe programs in cancer cells. *Cancer Cell* **6**: 625–630.
- van Golen CM, Soules ME, Grauman AR, Feldman EL. 2003. N-Myc overexpression leads to decreased β 1 integrin expression and increased apoptosis in human neuroblastoma cells. *Oncogene* **22**: 2664–2673.
- Varlakhanova NV, Cotterman RF, deVries WN, Morgan J, Donahue LR, Murray S, Knowles BB, Knoepfler PS. 2010. *myc* maintains embryonic stem cell pluripotency and self-renewal. *Differentiation* **80**: 9–19.
- Wakamatsu Y, Watanabe Y, Nakamura H, Kondoh H. 1997. Regulation of the neural crest cell fate by N-myc: Promotion of ventral migration and neuronal differentiation. *Development* **124**: 1953–1962.
- Wang K, Diskin SJ, Zhang H, Attiyeh EF, Winter C, Hou C, Schnepf RW, Diamond M, Bosse K, Mayes PA, et al. 2011. Integrative genomics identifies *LMO1* as a neuroblastoma oncogene. *Nature* **469**: 216–220.
- * Wiese K, Walz S, von Eyss B, Wolf E, Athineos D, Sansom O, Eilers M. 2013. The role of MIZ1 in MYC-dependent tumorigenesis. *Cold Spring Harb Perspect Med* doi: 10.1101/cshperspect.014290.
- Weiss WA, Aldape K, Mohapatra G, Feuerstein BG, Bishop JM. 1997. Targeted expression of MYCN causes neuroblastoma in transgenic mice. *EMBO J* **16**: 2985–2995.
- Welcker M, Orian A, Jin J, Grim JE, Grim JA, Harper JW, Eisenman RN, Clurman BE. 2004. The Fbw7 tumor suppressor regulates glycogen synthase kinase 3 phosphorylation-dependent c-Myc protein degradation. *Proc Natl Acad Sci* **101**: 9085–9090.
- Westermann F, Muth D, Benner A, Bauer T, Henrich K-O, Oberthuer A, Brors B, Beissbarth T, Vandesompele J, Pattyn F, et al. 2008. Distinct transcriptional MYCN/c-MYC activities are associated with spontaneous regression or malignant progression in neuroblastomas. *Genome Biol* **9**: R150.
- Wright JH. 1910. Neurocytoma or neuroblastoma, a kind of tumor not generally recognized. *J Exp Med* **12**: 556–561.
- Wu G, Broniscer A, McEachron TA, Lu C, Paugh BS, Beckfort J, Qu C, Ding L, Huether R, Parker M, et al. 2012. Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. *Nat Genet* **44**: 251–253.
- Yaari S, Jacob-Hirsch J, Amariglio N, Haklai R, Rechavi G, Kloog Y. 2005. Disruption of cooperation between Ras and MycN in human neuroblastoma cells promotes growth arrest. *Clin Cancer Res* **11**: 4321–4330.
- Yada M, Hatakeyama S, Kamura T, Nishiyama M, Tsunematsu R, Imaki H, Ishida N, Okumura F, Nakayama K, Nakayama KI. 2004. Phosphorylation-dependent degradation of c-Myc is mediated by the F-box protein Fbw7. *EMBO J* **23**: 2116–2125.
- Yancopoulos GD, Nisen PD, Tesfaye A, Kohl NE, Goldfarb MP, Alt FW. 1985. N-myc can cooperate with ras to transform normal cells in culture. *Proc Natl Acad Sci* **82**: 5455–5459.
- Zaizen Y, Taniguchi S, Noguchi S, Suita S. 1993. The effect of N-myc amplification and expression on invasiveness of neuroblastoma cells. *J Pediatr Surg* **28**: 766–769.
- Zhang C, Yan Z, Painter CL, Zhang Q, Chen E, Arango ME, Kuszpit K, Zasadny K, Hallin M, Hallin J, et al. 2009. PF-00477736 mediates checkpoint kinase 1 signaling pathway and potentiates docetaxel-induced efficacy in xenografts. *Clin Cancer Res* **15**: 4630–4640.
- Zhao X, D'Arca D, Lim WK, Brahmachary M, Carro MS, Ludwig T, Cardo CC, Guillemot F, Aldape K, Califano A, et al. 2009. The N-Myc-DLL3 cascade is suppressed by the ubiquitin ligase Huwe1 to inhibit proliferation and promote neurogenesis in the developing brain. *Dev Cell* **17**: 210–221.
- Zhu S, Lee J-S, Guo F, Shin J, Perez-Atayde AR, Kutok JL, Rodig SJ, Neuberger DS, Helman D, Feng H, et al. 2012. Activated ALK collaborates with MYCN in neuroblastoma pathogenesis. *Cancer Cell* **21**: 362–373.
- Zimmerman KA, Yancopoulos GD, Collum RG, Smith RK, Kohl NE, Denis KA, Nau MM, Witte ON, Toran-Allerand D, Gee CE. 1986. Differential expression of *myc* family genes during murine development. *Nature* **319**: 780–783.





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