Review

The N-myc Oncogene: Maximizing its Targets, Regulation, and Therapeutic Potential

Himisha Beltran

Abstract

N-myc (MYCN), a member of the Myc family of basic-helix–loop–helix–zipper (bHLHZ) transcription factors, is a central regulator of many vital cellular processes. As such, N-myc is well recognized for its classic oncogenic activity and association with human neuroblastoma. Amplification and overexpression of N-myc has been described in other tumor types, particularly those of neural origin and neuroendocrine tumors. This review outlines N-myc's contribution to normal development and oncogenic progression. In addition, it highlights relevant transcriptional targets and mechanisms of regulation. Finally, the clinical implications of N-Myc as a biomarker and potential as a target using novel therapeutic approaches are discussed. *Mol Cancer Res; 12(6); 815–22. ©2014 AACR.*

Introduction

The *N-myc* gene was first reported in 1983, when Schwab and colleagues (1) and Kohl and colleagues (2) discovered that there were a subset of human neuroblastoma cell lines harboring multiple copies of a DNA sequence related to the C-myc oncogene (MYC), and this region was designated N-myc (MYCN). Genomic amplification of N-myc resulted in overexpression of N-myc at the mRNA level, and rat embryo cells transfected with N-myc demonstrated oncogenic properties. These findings were first to bring attention to N-myc as a putative oncogene.

N-myc in normal development

N-myc belongs to the Myc family of proto-oncogenes, which are transcription factors of the basic-helix–loop–helix–zipper (bHLHZ) class and important for the regulation of gene expression associated with a range of cellular processes, including proliferation, growth, apoptosis, energy metabolism, and differentiation (3). Other Myc family transcription factors include C-myc and L-myc. Although similarly diverse in their function, Myc genes are separately regulated. C-myc is ubiquitous and highly expressed in most rapidly proliferating cells during development and continues to be expressed in dividing cells of adult tissues (4). N-myc has a restricted expression pattern: it is expressed during embryogenesis in pre-B cells, kidney, forebrain, hindbrain, and intestine, with highest expression in the developing brain. After embryonic development, N-myc is downregulated and N-myc is not significantly expressed in adult tissues. The L-myc gene is less well characterized, demon-

strates homology to N-myc and C-myc (5), and is expressed in both neonatal and adult lung tissue (4).

Because Myc proteins preferentially regulate the same sets of genes involved in differentiation and cell growth (i.e., translation, ribosome biogenesis, and metabolic processes), they can partially compensate for one another. For instance, mouse embryonic stem cells (ESC) homozygous for deletion of either *C-myc* or *N-myc* genes display morphology without aberrant proliferation or differentiation compared with wild-type ESCs (6, 7) and N-myc can functionally replace C-myc in murine development (8). However, regional morphology of the central nervous system differs in N-myc–null embryos from wild-type embryos, despite upregulation of C-myc (9). Therefore, further defining the Myc gene signatures that distinguish the targets of Myc proteins could provide insight into their tissue-specific roles in normal development and oncogenesis.

N-Myc plays an essential role in normal brain development. N-myc drives proliferation of granule neuron precursors derived from neuronal progenitor cells of the developing forebrain and hindbrain (10). Constitutive deletion of N-myc is embryonic lethal (6, 9), whereas conditional inactivation in neuronal progenitor cells leads to ataxia, behavioral abnormalities, and tremors that correlate with an overall 2-fold decrease in brain mass that disproportionately affects the cerebellum and the cerebral cortex, both of which show signs of disorganization. There exists severely compromised proliferation at the cellular level, including striking decrease in S phase and mitotic cells, whereas apoptosis is unaffected. In the neural crest, enhanced N-Myc expression facilitates proliferation of immature neuronal precursor cells at the expense of differentiation, and this can be stimulated by Sonic Hedgehog signaling (11).

Germline mutations that inactivate N-Myc cause Feingold syndrome, a rare autosomal dominant syndrome, associated with variable combinations of microcephaly, gastrointestinal atresia, limb abnormalities, and learning disability or mental retardation (12), a phenotype that may

Author's Affiliation: Weill Cornell Medical College, New York, New York

Corresponding Author: Himisha Beltran, Weill Cornell Medical College, 525 East 68th Street, Box 403, New York, NY 10005. Phone: 646-962-2072; Fax: 646-962-1603; E-mail: hip9004@med.cornell.edu

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be attributable to perturbance of normal neural stem and progenitor cell biology.

N-myc and human cancer

The original characterization of N-myc was soon followed by reports of its amplification in approximately 20% of cases of neuroblastoma, as well as by the observation that the degree of amplification correlated with advanced stage, unfavorable biologic features, and a poor outcome (13, 14). N-myc amplification was also associated with worse prognosis in otherwise favorable patient groups (13, 15–17). On the basis of these observations, N-myc gene copy number is standardly assessed by FISH in all clinical cases of neuroblastoma, and patients with N-myc amplification are stratified for more aggressive treatment (15, 18). This is a prototypic example of how identification of a prognostic genomic biomarker can directly translate into risk stratification and change patient management.

Aberrant amplification and/or overexpression of N-myc is not only seen in neuroblastoma, but has also been described in a number of other tumors types (Table 1). Although large-scale longitudinal studies assessing the prognostic value of N-myc have not yet been systematically reported for all tumor types, the presence of N-myc alterations has been associated with tumor aggressiveness and suggests a similar driving role in N-myc–amplified and/or overexpressed tumors.

N-myc is implicated in a subset of medulloblastoma (5% demonstrate MYCN amplification), is found in both Sonic Hedgehog–driven and non-Sonic Hedgehog–driven disease (19), and correlates with worse clinical outcomes (20).

Independent of amplification, activated Sonic Hedgehog and IGF/PI3K signaling in medulloblastoma can also upregulate N-myc through transcriptional upregulation and/or prevention of N-myc degradation (21). N-myc is implicated as a key driver of tumor initiation and progression in medulloblastoma preclinical models (19).

N-myc amplification also occurs in a subset of glioblastoma multiforme (22), is typically associated with forebrain rather than hindbrain tumors, and is rarely found in lower-grade gliomas and astrocytomas. Recently, genome-wide methylation analysis of both adult and pediatric glioblastoma multiforme identified recurrent functional mutations in the epigenetic modulator histone 3 gene (H3F3A; ref. 23), resulting in enhanced transcription of N-myc, which, in turn, promotes proliferation and suppresses differentiation (24). Therefore, targeting H3F3A-mutant tumors using therapeutic approaches to target N-myc may represent a novel therapeutic approach for a distinct subset of glioblastoma multiforme.

The majority of cases of retinoblastoma overexpress N-myc, and a small subset of these cases also harbors significant N-myc gene amplification (25–27). Although uncommon, N-myc amplification in retinoblastoma is independent of germline or somatic mutation of the retinoblastoma gene (RB1), and is associated with early onset (median age, 4.5 months), unilateral disease, and clinical aggressiveness (28). Therefore, N-myc–amplified retinoblastomas are thought to represent a distinct molecular subclass of nonfamilial retinoblastoma. N-myc overexpression, on the other hand, occurs in the majority of retinoblastoma cases and is not

Table 1. N-myc–amplified tumors

Tumor type	Frequency of N-myc alteration	Clinical implications	References
Neuroblastoma	Amplification in 20%	Poor prognosis, selection for aggressive treatment	Seeger et al. (13), Brodeur et al. (14), Look et al. (15), and Schneiderman et al. (17)
Medulloblastoma	Amplification in 5%	Poor prognosis	Aldosari et al. (92) and Swartling et al. (19)
Glioblastoma multiforme	Overexpression in a subset	Histone 3.3 mutations associated with overexpression of N-myc	Hodgson et al. (93) and Bjerke et al. (24)
Retinoblastoma	Amplification in <5%	Early onset, unilateral, nonhereditary, poor prognosis	Lee et al. (25) and Rushlow et al. (28)
Alveolar rhabdomyosarcoma	Amplification in 25%, overexpressed in 55%	Correlates with presence of PAX3-FOXO1 or PAX7-FOXO1 fusion genes, poor prognosis	Tonelli et al. (94)
Small-cell lung cancer	Amplification in 15%–20%	Poor response to chemotherapy, shorter survival	Nau et al. (32) and Funa et al. (34)
Prostate cancer	Amplification in 40% of neuroendocrine prostate cancer, 5% of prostate adenocarcinoma	Clinical aggressiveness	Beltran et al. (35) and Mosquera et al. (36)
Breast cancer	Overexpression in a subset	Correlates with poor prognostic features	Mizukami et al. (37)

a direct result of loss of regulatory control by RB1, but likely rather reflects an embryonal cell of origin (29). DNA amplification of N-myc occurs in a subset of rhabdomyosarcoma, a pediatric soft-tissue sarcoma, and is predominantly found in the alveolar subset and rarely in the more common embryonal subtype (30), although N-myc mRNA and protein expression is found in the vast majority of all rhabdomyosarcoma cases regardless of histology (31).

N-myc is amplified in 15% to 20% of small-cell lung cancers (32, 33) and is associated with poor response to chemotherapy, rapid tumor growth, and shorter survival (34). N-myc amplification rarely occurs in other lung cancer histologies. N-myc amplification also occurs in approximately 40% of neuroendocrine/small-cell prostate cancers, is commonly seen concurrently with amplification of the aurora kinase A gene (AURKA), and associated with poor prognosis (35, 36). In both small-cell lung and prostate cancers, N-myc amplification may occur early before the development of metastasis. Overexpression of N-myc has also been seen in a subset of breast cancers and correlates with poor prognostic features (37).

Functional targets of N-myc

Because of structural and sequence homology between the Myc family genes, the function and biochemical properties of Myc proteins are closely related. C-myc and N-myc can functionally replace one another in the appropriate context and share similar oncogenic properties (38). However, their functional roles seem to be tissue specific. For instance, C-myc is causally implicated in Burkitt lymphoma and translocation of the C-myc gene (*MYC*, chromosome 8q24) is pathognomonic. C-myc is also overexpressed in a wide range of malignancies including the majority of breast cancers, colon cancers, gynecologic cancers, hepatocellular carcinomas, and a variety of hematologic tumors, making C-myc one of the most commonly altered oncogenes in human cancer. L-myc is less well characterized but is often amplified and overexpressed in small-cell lung cancer (5). As mentioned, N-myc is primarily implicated in high-grade neuroendocrine tumors (neuroblastoma and small-cell carcinomas) and those arising from the neural system (neuroblastoma, medulloblastoma, and glioblastoma). Therefore, despite the fact that Myc genes may share related targets, they at the same time retain other, unique ones and are exclusively overexpressed in certain tumor types, suggesting that they may have independent and tissue-specific functions.

The fact that N-myc is normally not expressed in adult tissues but aberrantly expressed in tumors arising in the nervous system or dedifferentiated tumors such as small-cell carcinoma suggests origination or reversion to a neuronal stem cell–like phenotype in these tumors (associated with or driven by reactivation of N-myc). Therefore, understanding neuronal developmental pathways may help elucidate oncogenic pathways or downstream drivers of N-myc driven cancers.

N-myc is encoded by the *MYCN* gene on chromosome 2 (2p24). Genomic copy-number gain of *MYCN* is consistently associated with high levels of N-myc gene and protein expression (39), although overexpression can also occur in the absence of amplification. Functionally, N-myc is an oncogene; it can transform rat embryo fibroblasts (40) and plays a critical role in the pathogenesis of neuroblastoma. Studies in transgenic mice show that neural crest–specific expression of N-myc causes neuroblast transformation and the development of neuroblastoma (41). Expression of N-myc in nonamplified neuroblastoma cell lines can induce reentry of quiescent cells into the cell cycle and correlates with growth rate, motility, and cell attachment (42). The driving oncogenic role of N-myc is also enhanced by cooperating events such as *RAS* mutation due to Ras-mediated stabilization of N-myc and/or increase in N-myc translation (40, 43, 44). High expression of N-myc is associated with proliferation and induction of cell-cycle genes (45, 46) and also plays a role in regulation of apoptotic cell death (47).

There are a number of ways N-myc can turn on or off genes. One important mechanism is through heterodimerization with Max. N-myc is a 64-kDa nuclear protein with a N-terminus transcriptional activation domain and a C-terminus basic region helix–loop–helix leucine zipper motif (bHLH-Zip), which has DNA-binding activity and forms an obligate heterodimer with the bHLH-Zip protein Max (48, 49). Myc–Max complexes bind gene promoters by recognizing a DNA sequence, called E-box. N-myc preferentially binds the E-box CATGTG as well as the classic CACGTG. This in turn activates the transcription of downstream genes involved in diverse cellular functions. Under N-myc–amplified conditions, N-myc becomes less specific and can bind additional E-box motifs including CATTTG, CATCTG, and CAACTG (50). Global N-myc transcription factor binding analysis in neuroblastoma has also revealed modulation of N-myc binding through E-Box methylation. N-myc may also globally regulate gene expression through influencing chromatin structure via widespread histone acetylation and methylation across the genome (51). In addition, N-myc can also induce transcriptional repression of certain genes, independent of E-Box binding. This can involve recruitment of N-myc to target gene promoters by Miz-1 and disruption of the interaction between transcriptional complexes (52). A complex of Myc and Miz-1 bound to the promoter leads to transcriptional silencing, at least in part by recruitment of Dnmt3a (53). N-myc also directly interacts with another DNA methyltransferase EZH2 (54) and may repress target genes through DNA methylation. These data suggest that N-myc is also a key epigenetic regulator. In addition to binding to DNA, N-myc can also interact with several other proteins via its C-terminal domain including N-MYC interactor (NMI), transformation/transcription–associated protein (TRRAP), and TBP-interacting protein 49 (TIP49; ref. 18).

There are hundreds of proposed candidate target genes of N-myc, therefore, pinpointing the relevant oncogenic targets remains a significant challenge. Genome-wide gene expression analysis of 40 well-characterized human neuroblastomas and 12 cell lines with and without N-myc amplification has identified candidate N-myc target genes that

include genes associated with neural differentiation and those related to cellular proliferation (55). Expression of a subset of these genes was altered after transfection of a neuroblastoma cell line, SK-N-ER, with an N-myc–expressing gene construct when protein synthesis was inhibited. Most of the genes identified have consensus N-myc binding E-box sequences in their promoter regions, suggesting they represent direct targets. Similarly, short hairpin RNA (shRNA)–mediated silencing of N-myc in neuroblastoma cells has identified a 157 target-gene panel including those involved cell cycle, DNA repair, and neuronal differentiation genes; this panel has been associated with poor prognosis in patients with neuroblastoma. More recently, a similar set of N-myc–targeted genes has been observed in neuroendocrine prostate cancer, with upregulation of cell cycle and neuroendocrine type genes (35, 56). In prostate cancer, N-myc binds neuroendocrine and androgen-regulated gene promoters, suggesting direct involvement in neuroendocrine transformation. These observations support that the biochemical pathways of N-myc–amplified/overexpressed tumors may show overlap, regardless of tumor site of origin, and may serve a foundation for molecular classification and the development of molecular-based treatment approaches.

Because N-myc expression is associated with early stages of neural differentiation pathways and tumors often display reversion to a dedifferentiated developmental phenotype, it is intriguing to speculate that the N-myc–amplified cell may have stem cell–like characteristics. Coexpression of SOX9 with N-myc in cerebellar stem and progenitor cells has the ability to drive self-renewal, although neither SOX9 nor N-myc is able to drive self-renewal individually (11). N-myc can enhance the production of pluripotent and neural tumor stem cells and regulate the expression of ESC factors, including lif, klf2, klf4, and lin28b (57). N-myc also upregulates expression of the self-renewal protein BMI1 in neuroblastoma by directly binding to the E-Box sites within the promoter of BMI1 (58). These findings suggest potential mechanisms by which N-myc may contribute to dedifferentiation and maintenance of a pluripotent state.

Other implicated gene targets of N-myc include those involved in ribosome assembly and activity (59) and several cancer-associated genes/pathways, including MDM2 and TP53 (60), SKP2 (61), NLRR1 (62), Fyn kinase (63), DKK3/Wnt/β-catenin pathway signaling (61), and genes of the PI3k/Akt/mTOR pathway (64). N-myc can also inactivate Rb (through activating ID2 and also cooperating with RAS; ref. 65). N-Myc may also promote tumor angiogenesis through downregulation of leukemia inhibitory factor (LIF), a modulator of endothelial cell proliferation, which leads to activation of STAT signaling (66).

In addition to regulation of coding gene expression, N-myc has also been implicated as an important regulator of non–protein-coding gene expression. In neuroblastoma, N-myc can activate several microRNAs (mir-17-92, mir-221, mir-9, and mir-421) and long noncoding RNAs (T-UCRs and ncRAN) and repress others (mir-184 and mir-542-5p), which can contribute to the oncogenic phenotype through

regulation of downstream pathways involved in apoptosis, differentiation, epithelial-to-mesenchymal transition, and DNA damage response (67). Furthermore, transcriptome profiling of N-myc–associated miRNAs in human neuroblastomas has demonstrated prognostic significance (68).

N-myc can also indirectly upregulate the mitotic kinase AURKA through protein–protein interaction (69). Of a panel of 194 genes identified to be highly expressed in N-myc–amplified neuroblastomas compared with nonamplified tumors, shRNA targeting of each gene identified 17 genes that preferentially inhibited growth of N-myc–amplified versus nonamplified neuroblastoma cells (69). AURKA stood out among these genes, as it is now known that aurora A is highly expressed in N-myc–amplified tumors, can also mutually regulate N-myc protein stability, and is a synthetic lethal gene to N-myc. Importantly, the aurora A–N-myc interaction is potentially targetable using small-molecule inhibitors as described below.

Regulation of N-myc

Tight control of N-myc expression is an essential cellular mechanism for modulating N-myc function. There are a number of mechanisms for N-myc regulation; one is through control of N-myc turnover by protein degradation. N-myc protein turnover is cell-cycle dependent with N-myc degradation occurring during mitosis. Although N-myc has a short half-life of approximately 20 to 30 minutes, the extreme high steady-state levels (approximately 100-times normal) in amplified tumor cells ensures that the cells stay in cycle and do not enter G_0. Notably, the proposed 157 N-myc–regulated functional gene expression profile that associates with poor prognosis also associates with tumors with high nuclear N-myc protein levels, even in cases in which N-myc is not amplified or overexpressed at the mRNA level (64), supporting N-myc protein stability as one relevant mechanism for N-myc regulation (64). The ubiquitin ligase Fbxw7 normally targets N-myc for proteasomal degradation via the ubiquitin–proteasome system (70). This primarily occurs during the mitotic phase of the cell cycle and after phosphorylation of N-myc (S62 and T58) by the cell-cycle kinase cyclin B/CDK1 (70) and by glycogen synthase kinase-3β (GSK3β). Because GSK3β is inactivated by AKT, activation of the PI3K/AKT pathway results in stabilization of the N-myc protein (71, 72), and inactivation of the pathway using a PI3K inhibitor has been shown to reduce levels of N-myc (73). This is one therapeutically targetable pathway that may be relevant in other N-myc–driven and PI3K/AKT pathway implicated tumors as well.

AURKA is a mitotic kinase normally expressed during G_2 and mitosis, which can also act to stabilize N-myc by inhibiting Fbxw7-mediated degradation of the N-myc protein (69). AURKA is also frequently overexpressed and amplified in N-myc–amplified tumors such as neuroblastoma and neuroendocrine prostate cancer (35, 69), and cooperates with N-myc to promote tumor proliferation and oncogenic activity. N-myc–amplified neuroblastoma cells depend on high levels of AURKA expression for maintaining N-Myc function (69).

Another proposed mechanism for N-myc regulation includes translational regulation (74). For instance, activation of H-Ras through an oncogenic Ras mutation and other oncoproteins that hyperactivate the mitogen-activated protein kinase (MAPK) pathway can promote accumulation of N-myc by accelerating N-myc translation (74). This is a mechanism that is distinct from H-Ras/MAPK regulation of C-myc, which is primarily through protein stabilization. Various microRNAs (mir-34a, mir-101, and let-7) have also been documented to regulate N-myc by targeting mRNA, resulting in reduced N-myc expression and decreased proliferation of MYCN-amplified neuroblastoma cells (75, 76).

Detecting N-myc

The N-myc gene, MYCN, is located on the distal short arm of chromosome 2 (2p24). In cells with amplification, the extra copies typically reside as double minute or homogenously staining region amplicons (77). Additional genes may be coamplified with MYCN in a subset of neuroblastomas (DDX1, NAG, and ALK; ref. 78), but MYCN is the only gene that is consistently amplified from this locus. The ALK gene, which is nearby on chromosome 2p23, may also show a sporadic gain-of-function mutation in up to 8% of neuroblastomas (79). The magnitude of MYCN amplification varies, and typically ranges between 5 to 500+ copies per cell. Fluorescence In Situ Hybridization (FISH) is the standard assay used to detect MYCN amplification. It is technically reliable and can discern heterogeneous gene amplification among tumor cells in the same specimen (80).

The degree of DNA amplification often correlates with N-myc mRNA and protein expression, but overexpression of N-myc can also occur in the absence of MYCN amplification (64). However, to date, there is no strong correlation between mRNA or protein levels of N-myc and prognosis in neuroblastoma. Therefore, FISH remains standard rather than assessment of gene or protein expression for neuroblastoma. To date, N-myc status is not routinely tested in other tumor types.

Targeting N-myc

Because of evidence suggesting a causal role of N-myc in the development of neuroblastoma and a potential driver of other tumor types, and the fact that it is tumor specific and not expressed in normal tissue, the N-myc oncogene represents an attractive therapeutic target. However, similar to other transcription factors, selective inhibition of N-myc has been considered undruggable. Furthermore, the structure of N-myc is composed almost entirely of α helices with no surfaces for ligand binding.

There are a number of proposed approaches to indirectly target N-myc. Compounds that target the interface between Myc proteins and Max, identified through high-throughput screening of chemical libraries (e.g., Compound 10058-F4), have shown varied preclinical activity (81), but have generally been limited due to low potencies. Omomyc is a Myc-derived bHLHZip domain compound that interferes with Myc homodimerization by substituting four amino acids in the Myc zipper. It binds C-Myc and N-Myc, Max, and Miz-1 and prevents Myc binding to promoter E-boxes and transactivation of target genes while retaining Miz-1–dependent transrepression. Antitumor activity with oncomyc has been seen in vivo (82) and provides insight toward potentially developing clinical inhibitors that may target bHLH-ZIP proteins. Given the role of N-myc on DNA methylation, epigenetic therapies such as valproic acid and BL1521 have been tested in preclinical models and have shown promising results with induction of apoptosis, downregulation of N-myc, and changes in expression of N-myc target genes (83, 84).

Small-molecule inhibitors of acetyl-lysine recognition domain (i.e., bromodomain)– containing proteins can also target Myc transcription factors (85) by interfering with chromatin-dependent signal transduction and inhibiting Myc transcription. Recently, the bromodomain inhibitor, JQ1, was shown to target N-myc–amplified tumors by blocking N-myc–dependent transcription, impairing growth, and inducing apoptosis (86). Knockdown of the bromodomain protein BRD4 phenocopied these effects. Bromodomain inhibition also conferred a survival advantage in preclinical models of N-myc–amplified neuroblastoma, providing preclinical rationale for developing bromodomain inhibitors for patients with N-myc–driven tumors. Clinical investigation of bromodomain inhibitors for relapsed or refractory neuroblastoma is underway.

Identifying and targeting essential cofactors (such as AURKA) as a mechanism to destabilize N-myc or targeting upstream pathways of N-myc [such as phosphoinositide 3-kinase (PI3K)] may represent alternative mechanisms to indirectly target N-myc. Some of the available inhibitors of AURKA induce an unusually distorted conformation of its kinase domain, thus impairing its interaction with N-myc (87). AURKA inhibitors have shown preclinical activity in N-myc–amplified neuroblastoma and neuroendocrine prostate cancer (35, 66, 88), and are now undergoing phase II evaluation for these diseases. Recently, Bjerke and colleagues carried out a synthetic lethal screen to identify kinases that could represent a therapeutic target in N-myc–amplified pediatric glioblastoma multiforme (24). Out of 714 kinases tested, knockdown of CHK1 (checkpoint kinase 1) or AURKA promoted N-myc stability and reduced viability in N-myc–amplified cells. In a dose-dependent manner, the AURKA inhibitor VX-689 decreased levels of N-myc protein and cell viability; therefore, targeting AURKA or the aurora-A–N-myc interaction may be an effective approach for other N-myc–driven tumors as well. The CHK1 inhibitor CCT244747 has shown antitumor activity in neuroblastoma in vivo (89) and several CDK inhibitors are in various stages of clinical development.

Because phosphorylation of N-myc is directly regulated by Gsk3β, and indirectly by upstream signaling through the PI3K/Akt/mTOR pathway, targeting PI3K/Akt/mTOR may be an effective therapeutic approach. Furthermore, mTORC1 also downregulates PP2A, which normally dephosphorylates N-myc, targeting it for ubiquitination and promoting stabilization of N-myc. Akt is also commonly activated in N-myc–driven tumors. The PI3K/mTOR

inhibitor LY294002 has shown preclinical activity in neuroblastoma, which is rescued when cells are engineered to express T58/S62 phosphorylation site–deficient N-myc mutants (72, 90). The PI3K inhibitor NVP-BEZ235 has been shown to decrease angiogenesis and improve survival in N-myc neuroblastoma models (both *in vitro* and *in vivo*; ref. 91) N-myc expression was reduced in treated tumors, and evidence for both PI3K and mTOR inhibition was demonstrated, supporting the notion that PI3K inhibition can lead to inhibition or destablization of N-myc. Various inhibitors of PI3K, AKT, and TORC1/2 are in clinical development.

Conclusions

N-myc is an oncogenic transcription factor and a well recognized, clinically relevant prognostic biomarker for neuroblastoma. We now know that a subset of other solid tumors, especially small-cell carcinomas and neural-derived tumors, frequently harbor N-myc amplification, though systematic evaluation of N-myc status is not a standard practice for other tumor types. Because of the diverse range of targets of N-Myc, functional overlap with other Myc family proteins, and complex regulation, it remains challenging to elucidate which distinct targets of N-myc are driving tissue-specific oncogenesis and how regulation of these genes coordinate to promote tumorigenesis. However, continued investigation and developing mechanisms to directly or indirectly target N-myc are important steps toward improving outcomes for patients with N-myc–driven tumors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Himisha Beltran

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