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Trends in
Molecular Medicine

Review

Deciphering the MYCN-driven metabolic microenvironment of neuroblastoma

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Oncogenic MYCN drives aggressive disease in many cancers including neuroblastoma (NB). Metabolic reprogramming is essential to support cancer cell homeostasis and survival under nutrient- and oxygen-deprived conditions. MYCN directly reprograms many nodes of tumor-intrinsic metabolism, which have significant repercussions on the cells of the tumor microenvironment (TME), resulting in complex intercellular metabolic circuits that contribute to the immunosuppressive microenvironment of NB. These metabolic circuits are also regulated by the organismal and cellular circadian clock and host diet to further impact the TME and NB oncogenesis. This review discusses the mechanisms by which MYCN regulates the metabolic crosstalk between tumor, TME, and host, and provides evidence that therapeutic targeting of MYCN-reprogrammed metabolism can improve patient outcomes.

Highlights

Understanding how the *MYCN* oncogene reshapes both intratumoral and microenvironmental metabolic circuits should be considered in the design of novel metabolic therapeutic approaches for high-risk neuroblastoma (NB).

Targeting key MYCN-driven metabolic circuits may reprogram the tumor microenvironment (TME) of high-risk NB and improve immunotherapy approaches.

Identifying essential metabolic nodes of effector immune cells will inform metabolic-based strategies to optimize antitumor activity.

Systemic nutrition and circadian homeostasis crucially impact tumor metabolism and progression, and could be leveraged to develop novel therapeutic approaches for MYCN-driven cancers.

Rapid developments in methods to characterize the metabolic landscape at a single-cell resolution will enable more rationally designed metabolic therapies.

MYCN rewires tumor metabolism to promote tumor progression

The oncogene *MYCN* is amplified in 50% of high-risk NB tumors [1] and is the major driver of high-risk disease [2]. MYCN is a member of the MYC family of oncogene transcription factors that recognize EBOX motifs and regulate a variety of biological processes that support tumor growth [3]. Direct targeting of MYCN is an attractive therapeutic approach that has shown some promise. One recent approach, currently in a Phase 2 clinical trial, is the use of the miniprotein Omomyc (NCT06650514). However, despite some improvements, direct targeting of MYCN remains a major challenge because of the lack of a small-molecule binding domain, the presence of multiple coregulators, and severe toxicity upon prolonged inhibition [4]. Furthermore, even in the absence of *MYCN* amplification, patients who display a *MYCN* gene signature have poor clinical outcomes [5]. Therefore, targeting oncogenic pathways downstream of MYCN is crucial for improving high-risk NB patient outcomes. In tumors with activated MYCN the high demand for growth and biomass accumulation is achieved by metabolic reprogramming, including increased glucose and glutamine (Gln) uptake, and stimulation of mitochondrial biogenesis and nucleotide, lipid, and polyamine metabolism [6–10]. Targeting tumor metabolism via difluoromethylornithine (DFMO), an irreversible and FDA-approved ornithine decarboxylase 1 (ODC1) inhibitor, represents the most important success story for NB. ODC1 is a direct target of MYCN, and inhibition of polyamine biosynthesis with DFMO serves as proof of principle that inhibition of key MYCN-driven metabolic vulnerabilities can delay tumor progression and effectively improve event-free survival during maintenance therapy [11–15].

While much is known about how MYCN reprograms tumor-intrinsic NB metabolism, the effect of host metabolism on tumors is largely unknown. Solid tumors such as NB exist in a complex TME composed of resident cancer cells and other cancer-associated cells, including immune, endothelial, and fibroblast cells. These myriad cell types exhibit different metabolic dependencies in anatomically distinct sites, and can promote or inhibit tumor progression. Some of these metabolic dependencies are also controlled by whole-body metabolic homeostasis processes,

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including the molecular clock and the host diet, which, by altering the availability of circulating and TME nutrient levels, can impact both cancer and immune cells (Figure 1). Extrinsic mechanisms involving host-derived signals play a crucial role in tumor progression and response to therapy. An improved understanding of the metabolic interactions occurring in the TME will allow us to better employ the wide range of metabolic inhibitors that are currently available (Figure 2). This review discusses current understanding of the effects of MYCN metabolic reprogramming on extrinsic metabolism. We specifically highlight metabolic features that are unique to NB, such as polyamine metabolism, the opportunity for dietary interventions, and the molecular clock. We also discuss important metabolic nodes that may be relevant to MYCN-driven NB and should be considered for future studies as the field of metabolic therapies expands.

MYCN-reprogrammed metabolism disrupts the TME

The TME in high-risk *MYCN*-amplified (MNA) NB is highly immunosuppressive (Box 1). Metabolic crosstalk between NB and TME cells, specifically T cells, primarily takes place through two mechanisms: (i) metabolic antagonism, which involves cancer cells producing immunosuppressive metabolites that impair T cell functions, and (ii) metabolic competition, which occurs when metabolically demanding cancer cells limit T effector cell (Teff) functions by competing for nutrient availability (Figure 3).

Metabolic antagonism in the TME

Polyamines

MYCN transcriptionally activates *ODC1*, which encodes the rate-limiting enzyme that converts ornithine to putrescine, and thus promotes polyamine biosynthesis [15]. High *ODC1* expression predicts poor clinical outcome [15]. Because of the dependency of NB on polyamine metabolism, targeting polyamine synthesis, catabolism, and transport are effective therapeutic strategies to reduce NB burden [7]. Further, polyamine-addicted NB tumor cells fuel biosynthesis by depleting

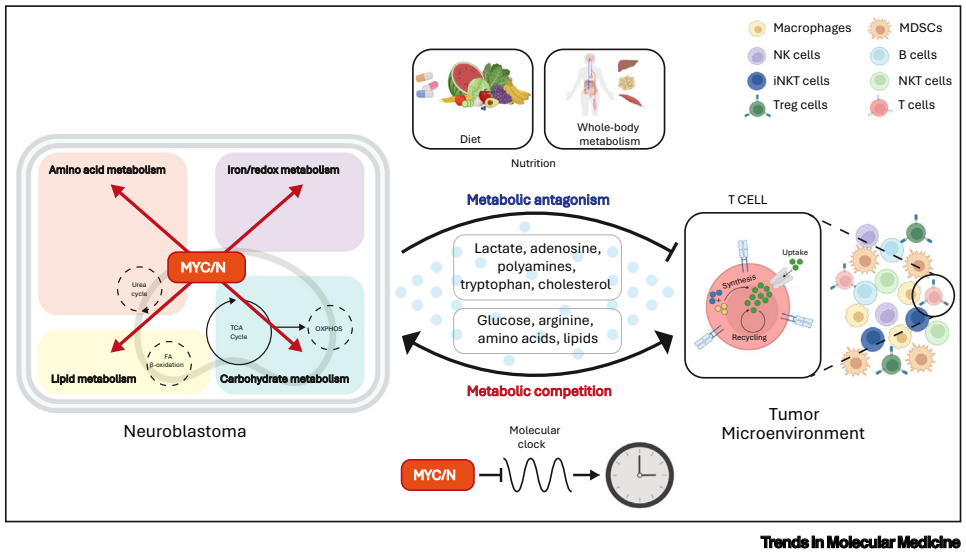

Figure 1. Regulation of intrinsic and extrinsic metabolism in neuroblastoma (NB). MYCN rewires the intrinsic metabolic pathways of NB cells which interact with the tumor microenvironment (TME) through two main mechanisms: (i) metabolic antagonism, and (ii) metabolic competition. Tumor-intrinsic and TME metabolism are further regulated by the molecular clock, which is disrupted by MYCN, as well as by nutritional intake and availability. Abbreviations: FA, fatty acid; iNKT cells, invariant natural killer T cells; MDSCs, myeloid-derived suppressor cells; NK cells, natural killer cells; NKT cells, natural killer T cells; OXPHOS, oxidative phosphorylation; TCA cycle, tricarboxylic acid cycle; Treg cells, regulatory T cells.

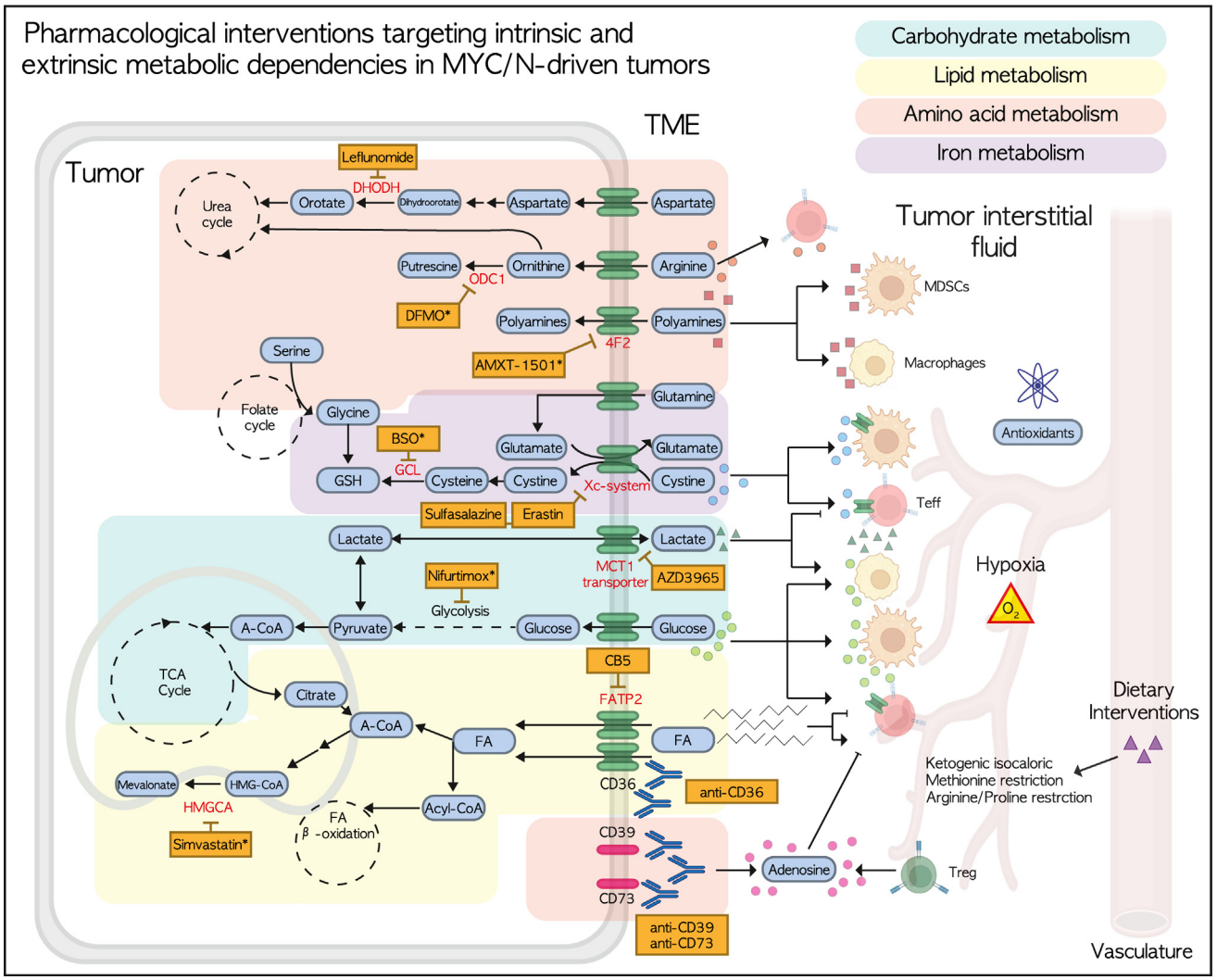

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Figure 2. Pharmacological interventions targeting intrinsic and extrinsic metabolic dependencies in MYCN-driven tumors. MYCN rewires multiple facets of intratumoral metabolism. Both tumor-intrinsic and host metabolism modulate the microenvironmental metabolic landscape, including immune cell metabolism. Metabolites are represented in light-blue squares; enzymes that are specifically regulated by MYCN are indicated in red; orange squares represent therapeutically promising compounds that target key metabolic enzymes. Many of these compounds, indicated by *, are currently in clinical trials for neuroblastoma (NB); results are available for the trials listed in italic font, and trials in bold font are associated with immunotherapy agents. Nifurtimox (glycolysis inhibitor): NCT00486564 (with cyclophosphamide and topotecan), *NCT00601003* (with cyclophosphamide and topotecan). Simvastatin (HMG-CoA reductase inhibitor): NCT02390843 (with cyclophosphamide and topotecan). DFMO (ODC1 inhibitor): *NCT02395666*, NCT04301843 (with etoposide), NCT02679144, NCT02030964 (with cyclophosphamide, topotecan, and colecoxib), *NCT02139397* (with bortezomib), **NCT02559778 (with induction chemotherapy and immunotherapy)**, NCT03581240, *NCT01059071* (with etoposide), NCT06465199 (with AMXT-1501), **NCT03794349 (with irinotecan, temozolomide, and dinutuximab)**. AMXT-1501 (SLC3A2 inhibitor): NCT06465199 (with DFMO). BSO (buthionine sulfoximine, GCL inhibitor): NCT00002730 (with melphalan), NCT00005835 (with melphalan). Abbreviations: A-CoA, acetyl-CoA; DFMO, difluoromethylornithine; FA, fatty acid; GSH, glutathione; MDSCs, myeloid-derived suppressor cells; TCA cycle, tricarboxylic acid cycle; Teff, effector T cell; TME, tumor microenvironment; Treg, regulatory T cell.

arginine (Arg), which has detrimental effects on Teff functions [16]. While DFMO is a NB tumor-targeted agent, it also has immunomodulatory effects by stimulating T cell and natural killer (NK) cell-mediated responses, especially when combined with polyamine uptake inhibitors [11]. However, despite the success of DFMO in NB patients, the role of polyamine metabolism in T and NK cells remains controversial. Polyamines are crucial for T cell activation, proliferation,

Box 1. The tumor microenvironment (TME) of NB is profoundly dysfunctional

The TME of high-risk MNA NB is highly immunosuppressive because of low MHC-I expression, limited infiltration of $CD8^+$ Teff cells, NK cells, NKT cells, and iNKT cells and high infiltration of M2 proinflammatory macrophages and MDSCs [131]. One of the major challenges to antitumor immunity is progressive loss of functional $CD8^+$ Teff cells within the TME. T cell function and survival in solid tumors are shaped by nutrient availability (see Figure 3 in the main text). Nutrient limitation makes $CD8^+$ Teff cells more dysfunctional by reducing self-renewal capacity, upregulating inhibitory receptors, and impairing mitochondrial function [132–134]. In NB, TILs have reduced cytotoxic activity, express markers of exhaustion (including PD-1 and TIM-3), localize distantly from tumor cells, and display dysfunctional gene signatures that predict poor patient outcomes [135,136]. Effective immunotherapies for NB will need to overcome this suppressive TME.

and T helper cell (T_H) subset differentiation [17]. However, upregulated ODC1 and polyamine levels can also disrupt T_H1 interferon (IFN)-γ production and induce Forkhead box protein P3 (*FoxP3*) gene expression to promote T cell dysfunction [18]. Further, spermidine inhibits T cell receptor (TCR) signaling to block T cell proliferation and cytokine production [19]. These competing phenotypes suggest a tissue-specific or state-specific role for polyamine metabolism in tumor-infiltrating lymphocytes (TILs) that could be better elucidated with spatial metabolomics (Box 2). In the myeloid compartment, inhibition of polyamine synthesis enhances M1 macrophage phenotypes [20] and impairs myeloid-derived suppressor cell (MDSC) activity by downregulating

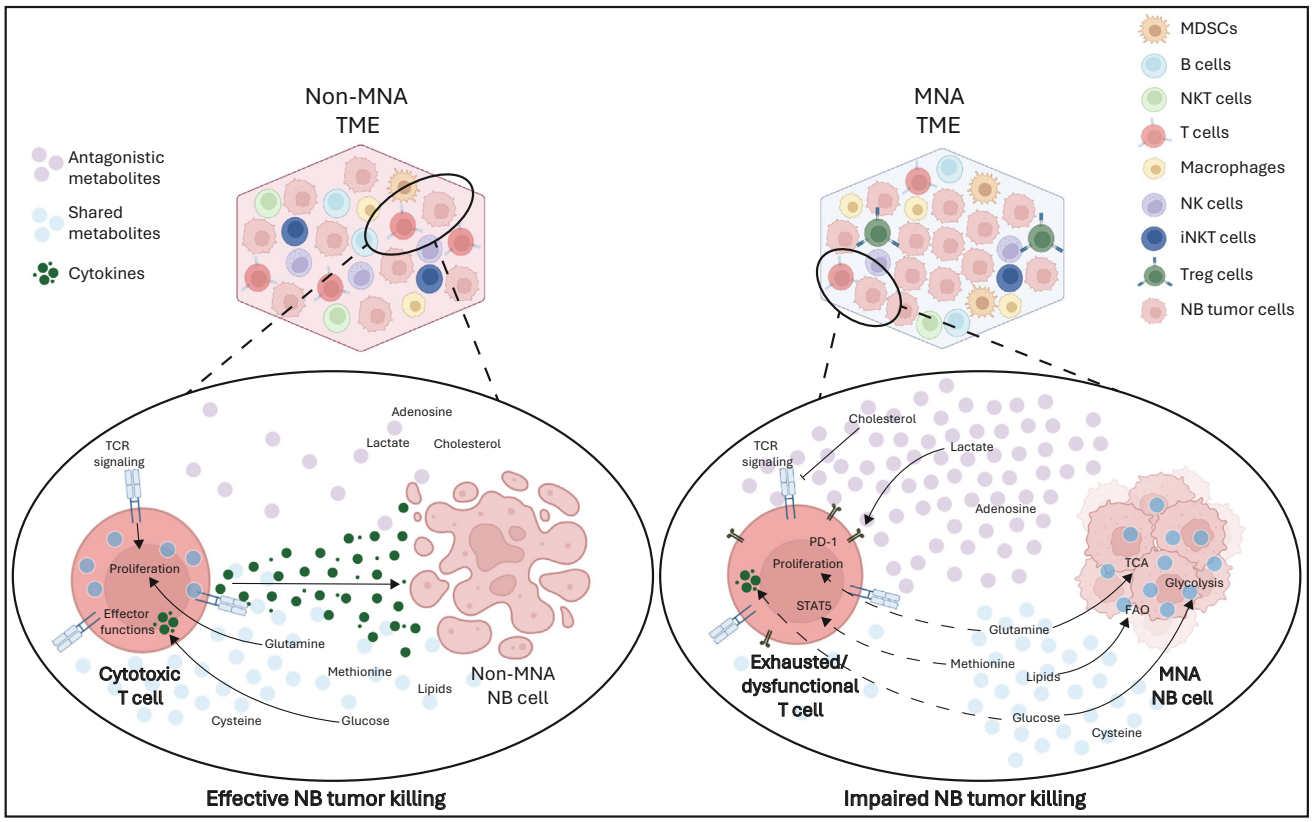

Figure 3. The metabolic landscape and tumor microenvironment (TME) of *MYCN*-amplified (MNA) and non-MNA neuroblastoma (NB). MYCN rewires cellular metabolism to promote NB cell consumption of exogenous metabolites that, when deprived from T cells, induce T cell exhaustion and dysfunction. MNA NB cells also secrete immunosuppressive metabolites into the TME that can further hinder T cell function. Alternatively, non-MNA NB cells do not regulate the metabolic landscape in this same way, and are thus more subject to cytotoxic T cell infiltration and effector function, which can induce NB cell death. Abbreviations: FAO, fatty acid oxidation; iNKT cells, invariant natural killer T cells; MDSCs, myeloid-derived suppressor cells; NK, natural killer cells; NKT, natural killer T cells; TCA, tricarboxylic acid cycle; TCR, T cell receptor; Treg cells, regulatory T cells.

Box 2. Single-cell and imaging technologies for metabolic investigation

To study TME metabolism, modulation of individual metabolite levels in the media is a well-controlled and feasible method. However, many of these studies have not used physiologically relevant metabolite concentrations, resulting in 'all or nothing' experiments. Recent advances in tumor interstitial fluid isolation will facilitate the identification of physiological metabolite levels within the TME to better inform these studies [137]. Further, the design of effective and rationale therapies for patients requires appropriate *in vivo* modeling of TME metabolic interactions and a deeper understanding of tumor metabolism at the individual patient level. *In vivo* metabolic analyses generally rely on mass spectrometry approaches using bulk tumor tissue and therefore fail to appropriately capture the real-time dynamics of metabolism at single-cell resolution. Novel techniques have been developed to study single-cell immunometabolism, including antibodies for cytometry-based metabolic profiling, nutrient-uptake assays and single-cell fluxomics tools for assessing the temporal regulation, and standardized analysis pipelines for single-cell metabolic data and multiomic data integration [138]. Although these tools are still under development, the field of immunometabolism has progressed significantly through the use of metabolomic imaging which is capable of resolving metabolites at the near single-cell level with intact spatial organization. Metabolic imaging techniques, such as positron emission tomography (PET) [139] and hyperpolarized nuclear magnetic resonance (NMR) [140] can help to dissect the intratumoral and TME metabolic landscapes *in vivo*. These strategies, combined with refined single-cell imaging techniques such as single-cell profiling and imaging of cell energy metabolism (SPICE-Met) [141], will better illuminate the inter- and intratumoral heterogeneity of NB and MYCN-regulated metabolic crosstalk within the TME to enable the design of more precise therapies.

arginase 1 (ARG1) [21]. ODC1 inhibition results in ornithine accumulation, which can further inhibit ARG1 activity [22]. Importantly, DFMO sensitizes tumors to PD-1 blockade by improving $CD8^+$ T cell viability and effector functions [23], and directly enhances the efficacy of adoptive T cell therapy (ACT) [21]. While the effects of polyamine metabolism on $CD8^+$ Teff cells require further elucidation, inhibition of polyamines via DFMO may allow dual targeting of both tumors and immunosuppressive myeloid cells. Ongoing clinical trials are evaluating the effect of DFMO in combination with anti-GD2 immunotherapy in NB patients (NCT03794349) and will better elucidate the efficacy of combining polyamine metabolism inhibition with immunotherapy.

Lactate

During their excessive growth, both NB and stromal cells export lactate, a byproduct of aerobic glycolysis, via mononcarboyxlate transporter 1 (MCT1) [24,25]. Owing to their preferential expression of MCT11 [26], a lactate importer, lactate blocks $CD8^+$ Teff functions by suppressing pyruvate carboxylase activity [27] and NFAT expression [28]. Further, lactate represses NK cell cytotoxic activity [28] and promotes increased MDSC infiltration [29] and M2 macrophage polarization [30]. MYC directly upregulates MCT1 [31] and targeting MCT1 reduces lactate secretion; however, in NB, MCT4 can be upregulated under hypoxia to compensate for MCT1 inhibition [25]. Therefore, reducing lactate levels by other means, such as by blocking the conversion of pyruvate into lactate via lactate dehydrogenase A (LDHA), may be crucial for targeting cell resistance mechanisms to metabolic inhibitors. Encouragingly, LDHA inhibition promotes NK and T cell infiltration [28] and activity [29] and blocks MDSC suppressive activity [29]. Thus, the combination of metabolic inhibitors that target multiple arms of lactate production, secretion, extracellular acidification, and its effects on signaling may be crucial to inhibit its immunosuppressive activity.

Adenosine

Immunosuppressive adenosine is produced by the ectonucleotidases CD39 and CD73 from extracellular ADP and AMP [32,33]. In NB, CD73 is highly expressed and predicts poor outcomes in MNA stage 4 patients [34]. CD73 further promotes mesenchymal-like phenotypes and blocks NK cell-mediated killing [34]. While both tumor cells and regulatory T cells (Tregs) produce extracellular adenosine [35], activated T cells upregulate their expression of equilibrative nucleoside transporter 1 (ENT1) to consume exogenous adenosine [36], and thus blockade of adenosine production or uptake may represent a promising strategy. In models of pancreatic ductal adenocarcinoma (PDAC), inhibition of CD73 controls tumor growth and enhances T cell infiltration and activation [37], and may be of therapeutic interest for NB. Interestingly, CD39 has also been

identified as a marker of both T cell and chimeric antigen receptor (CAR)-T cell exhaustion [38,39], suggesting that exhausted T cells can further contribute to immunosuppression by producing adenosine. Future efforts to target CD39 and CD73 in NB, perhaps by immune checkpoint blockade (ICB) or via CAR-T cell enhancement (Box 3), may overcome adenosine-mediated suppression.

Lipids

NB cells exhibit dysregulated lipid metabolism that supports tumor growth and metastasis [40]. Lipids are ubiquitous molecules that function as energy sources, signaling molecules, and structural membrane elements. MYC directly regulates the expression of fatty acid (FA) biosynthetic enzymes to promote *de novo* lipid synthesis and desaturation [41]. Importantly, inhibition of *de novo* lipid synthesis blocks NB proliferation and promotes tumor cell differentiation [40]. Although the requirement for FAs is mostly met by *de novo* synthesis, our laboratory has shown that FA uptake from the microenvironment [through transporters such as fatty acid transport protein 2 (FATP2)] is also a crucial source of lipids [10]. While lipids play a central role in the metabolism and function of T cells [42,43], excessive accumulation of lipids in $CD8^+$ T cells impairs their antitumor properties. The TME is a lipid-rich locus (both of FAs and cholesterol) that benefits tumor growth and impairs T cell functions [44,45]. In pancreatic cancer, intratumoral $CD8^+$ T cells accumulate long-chain and very long-chain FAs which promote T cell dysfunction and lipotoxicity [46]. Similarly, systemic or $CD8^+$ T cell-specific deletion of the FA transporter gene *Cd36* rejuvenates the antitumor functions of infiltrating $CD8^+$ T cells by blocking T cell uptake of oxidized low-density lipoproteins (OxLDLs) and lipid peroxidation [47]. Importantly, inhibition of FA synthase (FAS) induces $CD8^+$ T cell activation and protects $CD4^+$ Teff cells from restimulation-induced cell death [48]. In addition, high FAS and *de novo* lipogenesis contribute to the functional maturation of Tregs and support the immunosuppressive functions of neutrophils, suggesting that this class of inhibitors may have therapeutic potential in cancer immunotherapy [49,50]. Similarly, MYC transcriptionally regulates the expression of the squalene epoxidase (*SQLE*) gene which encodes the rate-limiting enzyme of cholesterol biosynthesis [51] and maintains NB stemness and tumorigenicity [52]. Free cholesterol can be released into circulation and esterified by acetyl-CoA acyltransferase (ACAT) enzymes [53]. Cholesterol levels, which are higher in TILs, upregulate endoplasmic reticulum (ER) stress responses to induce immune

Box 3. Metabolically-enhanced CAR-T cells are a promising therapeutic intervention

A major advance in therapy for NB is the use of anti-GD2 CAR-T cells [142]. Despite some promising results, CAR-T cells in solid tumors fail for several reasons, including on-target/off-tumor toxicities, poor trafficking, insufficient persistence, exhaustion, and tumor antigen heterogeneity [143]. CAR-T cell differentiation, activation, and function are intricately linked to their metabolism, and the selection of specific costimulatory signaling domains can have divergent effects on CAR-T cell metabolism [144]. However, the ability to manipulate the metabolic environment or genetically engineer T cells *ex vivo* has potential to metabolically enhance and reprogram cell therapies. Supplementation of specific metabolites such as NAC during CAR-T cell product generation can promote stem-memory T cell phenotypes and enhance tumor control. Although these phenotypes have been shown to persist up to 35 days, genetic manipulation of essential metabolic enzymes may offer a longer-term solution to improve CAR-T cell metabolic fitness in the nutrient-depleted TME. For example, CAR-T cell products with enhanced glycolysis display improved antitumor activity upon infusion into patients [95], and constitutively active AKT/GLUT1 in anti-GD2 and anti-CD19 CAR-T cells enhances antitumor activity [145,146]. However, 2-deoxyglucose (2-DG) treatment during CAR-T cell expansion results in less differentiated and more stem-like memory CAR-T cell products [147] that are more cytotoxic and have improved self-renewal and persistence [148]. In addition, CAR-T cells engineered to express Arg biosynthesis enzymes such as argininosuccinate synthase or ornithine transcarbamylase, or Cys metabolism enzymes such as cystathionine-γ-lyase or xCT, have enhanced antitumor activity [149–151]. Alternatively, arming CAR-T cells with enzymes that can break down or block suppressive metabolite consumption and signaling can prevent dysfunction. For example, CAR-T cell overexpression of adenosine deaminase (ADO) metabolizes adenosine into inosine to prevent exhaustion and develop T cell stem phenotypes [39]. Although our imagination is limitless, considerable effort will be necessary to identify vital enzymes that would best metabolically enhance CAR-T cells for their specific tumor target and localization [152].

checkpoint expression and T cell exhaustion [45]. In addition, cholesterol sulfate blocks TCR signaling [54], and inhibition of cholesterol esterification before CAR-T cell transduction increases CAR-T cell cytotoxic activity [55]. These data indicate that modulation of both systemic and T cell-specific lipid levels can enhance immunity.

Other emerging pathways

An essential hallmark of NB, because of its neural crest and adrenal chromaffin cell origins, is excessive production of catecholamines [56]. Norepinephrine treatment blocks intratumoral $CD8^+$ T cell infiltration by inhibiting CXCL9 (CXC motif chemokine ligand 9) levels [57] and IL-2 and IFN-γ expression in central and effector memory $CD8^+$ T cells [58]. Further, inhibition of the (nor)epinephrine receptor β2-AR blocks MDSC signaling, recruitment, and immunosuppressive activity [59]. The propensity of NB tumors to produce high levels of (nor)epinephrine and the ability of these molecules to promote immunosuppression highlight a crucial role of catecholamine metabolism beyond serving as a NB biomarker.

Additional metabolic pathways that are regulated by MYC/N and have immunosuppressive functions may have important, understudied roles in NB. Gamma-Aminobutyric acid (GABA), a neurotransmitter produced via glutamate that has a prognostic role in NB [60], blocks T cell IFN-γ and tumor necrosis factor (TNF)-α production [61] and inhibits antigen-presenting cell (APC) inflammatory responses [62]. Silencing GABA profoundly inhibits lung cancer growth in immunocompetent mice, increases T cell infiltration, and resensitizes tumors to ICB [61]. Because both glutamate and polyamines are known GABA precursors, it is likely that MYCN controls GABA metabolism, and this may be an important target in NB.

MYCN also directly regulates mitochondrial respiration and the electron transport chain (ETC) to generate sufficient energy for growth [63]. Importantly, deletion of ETC complex II subunits can promote succinate accumulation, which upregulates MHC-I and increases T cell infiltration [64]. Autophagy, which maintains metabolic homeostasis and nutrient recycling, is inhibited by MYC activity [65] and regulates antigen expression and MHC-I [66]. NB is characterized by low levels of MHC-I [67], which masks tumor cells from T cell recognition; ETC inhibition or autophagy activation in NB may thus enhance T cell recognition and tumor cell killing.

Last, both tryptophan (Trp) and its carboxylated byproduct kynurenine are major immunosuppressive metabolites in a variety of cancers [68]. Ectopic MYC expression increases Trp and its conversion into kynurenine by regulating the expression of Trp transporters and arylformamidase [69]. Kynurenine promotes T cell dysfunction [70] and may share a similar suppressive function in MNA NB.

Overall, MYCN-driven tumors and TME cells produce several metabolites with immunosuppressive properties, suggesting that targeting specific metabolic dependencies may also reinvigorate antitumor immunity. Future efforts should elucidate the potential consequences of overactivated immune cells when inhibiting these pathways and their implications for therapy response and toxicities.

Metabolic competition in the TME

Polyamine and arginine

NB tumors consume exogenous polyamines and Arg, a non-essential amino acid (AA) that is used for polyamine biosynthesis [16]. NB tumors also have arginase activity and tumor supernatants impair T and CAR-T cell proliferation in an arginase-dependent manner [16]. Other TME cells such as M2 macrophages and polymorphonuclear (PMN)-MDSCs also upregulate ARG1 to

deplete exogenous Arg and inhibit T cell proliferation [71]. While the role of exogenous polyamines on T cell function remains unclear, Arg maintains T cells in an undifferentiated, central memory state, supports enhanced T cell survival, and promotes improved ACT antitumor responses [72]. Future efforts to delineate the dependency of T cells on exogenous Arg and polyamines will be essential.

Amino acids

While NB cells can *de novo* synthesize Gln, exogenous Gln is also essential and MYCN directly controls the expression of the Gln transporter ASCT2 (solute carrier family 1 member 5, *SLC1A5*) gene [63,73]. T cell activation upregulates the expression of Gln transporters and biosynthesis enzymes to support cell proliferation, differentiation, and IL-2 and IFN-γ secretion [74]. Moreover, increased availability of Gln in the tumor interstitial fluid enhances Teff functions and ACT efficacy [75,76]. PD-L1 ligation with PD-1$^+$ T cells blocks Gln uptake via SNAT1/SNAT2 [77], thus anti-PD-1 or anti-PD-L1 therapies should enhance Gln uptake and metabolically reinvigorate T cells. Despite this evidence, *ex vivo* inhibition of Gln metabolism during CAR-T cell production was found to enforce a switch towards oxidative metabolism and alter the epigenetic landscape and transcriptional programs towards naïve and long-lived central memory T cell subset phenotypes that enhance antitumor activity [78]. Future studies will need to elucidate the time- and dose-specific effects of Gln perturbation on T cell, ACT, and CAR-T cell fates.

Cyst(e)ine [$Cys_{(2)}$] is considered to be a conditionally essential AA which contributes to protein and glutathione (GSH) synthesis. NB is exceptionally dependent on $Cys_{(2)}$; MYCN transcriptionally activates the xCT $Cys_{(2)}$ importer subunits *SLC3A2/SLC7A11*, and exogenous deprivation or pharmacologic inhibition of xCT induces NB cell death via ferroptosis [79,80]. T cells also upregulate their expression of xCT upon stimulation [81] to support proliferation [82]. Thus, supplementation of *N*-acetyl cysteine (NAC) in *ex vivo* stimulated T cells for ACT protects them from reactive oxygen species (ROS)-induced DNA damage and enhances tumor control [83]. This shared dependency suggests that pretreating NB tumors with xCT inhibitors could potentially increase the availability of $Cys_{(2)}$ in the TME for T cell consumption following ACT.

As with many pediatric cancers, NB has a dysregulated epigenetic landscape [84]. Methionine (Met), an essential AA, produces the universal methyl donor *S*-adenosyl-methionine (SAM). *N*6-methyladenosine (m^6A) modification of mRNA and protein methylation are essential for T cell proliferation, differentiation, and effector functions [85–87]. Because Met and SAM regulate T cell epigenetics, understanding how epigenetic inhibitors differentially impact tumors and Teff functions is imperative.

Glucose

MYCN promotes GLUT1 (solute carrier family 2 member 1, *SLC2A1*) expression for the consumption of exogenous glucose [88]. Similarly, T cell activation promotes AKT signaling and GLUT1 expression for T cell glucose consumption [89–91] which is essential for T cell growth, IFN-γ production, and differentiation into effector and memory phenotypes [89]. Tumors with enhanced glycolytic activity impair T cell glycolysis, promote T cell PD-1 expression, and block IFN-γ production to induce hyporesponsive phenotypes [92]. Monocytic (M)-MDSCs and tumor-associated macrophages also excessively consume exogenous glucose from the TME [93], and sites of NB metastasis, such as the bone marrow, are prone to glucose competition by tumor cells, immune cells, and bone-derived cells [94]. Therefore, boosting glucose availability to T cells specifically may enhance their antitumor activity in both primary and metastatic sites. While systemic supplementation of metabolites to patients may unknowingly promote tumor

progression, metabolic enhancement of CAR-T cells during production and infusion may circumvent this limitation and could represent a promising therapeutic approach (Box 3) [95].

Lipids

MYCN promotes NB cell dependency on FA uptake [10]. While lipid accumulation can promote T cell dysfunction, lipids are crucial for membrane synthesis and T cell expansion. Teff cells acquire FAs from the TME whereas memory T cells employ *de novo* lipid synthesis and hydrolysis to support fatty acid oxidation (FAO) [96]. Further, linoleic acid exposure potentiates memory T cell phenotypes and enhances ACT and CAR-T therapies [97]. However, FAO and lipid accumulation are also associated with naïve and undifferentiated T cell phenotypes and Tregs [98]. Future work will need to elucidate the lipid species and optimal conditions for supplementing lipids to CAR-T cell therapies to encourage T cell expansion while preventing dysfunction.

MYCN and host metabolism: opportunities for nutritional interventions and the effects of the molecular clock

Changes in nutrient availability have profound effects on tumorigenicity and immune cell functions. However, the molecular mechanisms of how diet impacts NB metabolism, including its interactions with the TME, remain largely unknown. This highlights the possibility that defined diets can be leveraged to counter MYCN-driven metabolism, and emphasizes the need to systematically assess the functional significance of different diet compositions on cancer and host metabolism.

Nutritional interventions

Because NB is generally an embryonic malignancy, exploration of maternal nutritional interventions to prevent disease development is warranted. Prenatal folic acid supplementation is associated with reduced NB incidence [99]. Because folic acid is crucial for DNA synthesis/repair and generation of methyl donors, such as Met and SAM, folic acid insufficiency leads to DNA hypomethylation and subsequent genomic instability that can potentiate carcinogenesis [100]. The effects of folic acid on Teff functions via methyl donors also need to be elucidated. Prenatal multivitamin supplementation is also associated with a lower risk of NB [101], in part because of the antioxidant functions of vitamins A, C, and E in reducing ROS activity [102]. GSH acts as a major antioxidant by eliminating ROS and inhibiting lipid peroxidation, and can thus suppress tumor growth [102]. However, GSH can also directly protect cancer cells from oxidative stress [103], induce chemoresistance [104], and promote metastasis [105]. These competing roles of antioxidants highlight the crucial need to understand the impact of redox regulation at specific concentrations on cancer development and progression in context-specific models.

Docosahexaenoic acid (DHA) is a crucial FA for infant development [106]. DHA delayed NB onset in an immunocompromised mouse xenograft model; however, it failed to prevent tumor development in a tyrosine hydroxylase (TH)–MYCN genetically engineered mouse model (GEMM) [107], suggesting that the suppressive TME of NB may influence the response to DHA. MYCN directly suppresses DHA synthesis by downregulating the expression of ELOVL2 (FA elongase 2), a key FA elongase that synthesizes very long-chain FAs [108]. However, the contribution of specific dietary fats to NB remains largely unknown and the role of a high-fat diet (HFD) and maternal obesity in NB development and prevention will need to be investigated. Because specific FAs may function as immunomodulators (by reprogramming $CD8^+$ T cell function) in premalignant cells, maternal diet will likely influence multiple facets of NB development. In addition to FA modulation, reduced glucose consumption with an isocaloric ketogenic diet (KD) combined with caloric restriction showed antitumor activity in both MNA and non-MNA NB xenografts [109]. Moreover, a medium-chain FA-containing KD was more effective than a long-chain FA-

containing diet in controlling tumor growth [110], suggesting that specific FA components also need to be considered when designing KD interventions. A KD can enhance the efficacy of both standard chemotherapy and targeted agents, including PI3K inhibitors, by lowering blood insulin and deactivating the insulin receptor in solid tumors [111]. In addition, a KD resensitizes murine melanoma tumors to anti-PD-1 and anti-CTLA-4 therapies by enriching $CXCR3^{+}CD8^{+}$ T cells [112], suggesting that reduced glucose consumption can also reactivate the TME. The interaction between KD diets and targeted agents or immunotherapy in the context of NB warrants further investigation.

Dietary manipulation of some AAs is also a promising approach to counter tumor metabolism [113]. MYCN drives Met and serine (Ser) metabolism by demanding a supraphysiological supply of Cys to maintain redox homeostasis and protein synthesis [80]. Dietary Met restriction (MR) has shown anticancer activity in numerous preclinical studies and has synergistic effects with radio/chemotherapy [114] and anti-PD-1 immunotherapy [115]. Importantly, MR can also limit the biosynthesis of polyamines, and thus holds promise in polyamine-dependent cancers such as NB. Last, because Met metabolism shapes T helper cell responses [116] and drives T cell exhaustion [117], MR could also condition the nutrient milieu of NB TME. However, the role of MR in antitumor immunity is complex because Met supplementation has also been shown to improve immune functions in several syngeneic models [86]. Similarly, double Ser and glycine (Gly) restriction has also shown antitumor activity in a variety of cancers by restricting protein, nucleic acid, and lipid biosynthesis [113]. Interestingly, dietary restriction of Arg and proline (Pro), two upstream AA substrates for ornithine and polyamine biosynthesis, augments DFMO-mediated tumor polyamine depletion, promotes NB tumor differentiation, and enhances survival in the TH-MYCN GEMM [118], suggesting that the use of defined diet–drug combinations is a clinically viable approach for NB therapy. Overall, understanding how AA restriction could be therapeutically exploited in NB remains to be determined.

Last, diet can directly influence the composition and functionality of the gut microbiota. NB patients present specific microbial features [119], suggesting that pre- and probiotics may be utilized for adjunct NB therapy. Overall, these findings indicate that nutrients can be utilized to enhance anticancer efficacy by targeting tumor metabolism, the TME, and the microbiome. Currently, most nutritional studies are limited to adult cancers. Because pediatric cancers differ in their pathological features from adult cancers and could be influenced by maternal nutrition, additional preclinical nutritional interventions and mechanistic investigations in specific pediatric cancer contexts will be necessary to better understand how nutrition can support current treatment strategies.

The role of the molecular clock

Host metabolism follows circadian rhythms that are generated endogenously and maintained by tissue-specific gene networks under the transcriptional control of molecular clock regulators. NB has a dysfunctional clock machinery. Our laboratory has shown that MYCN drives NB oncogenesis and metabolic reprogramming by inhibiting the core circadian clock component brain and muscle ARNT-like 1 (BMAL1), which functions as a metabolic checkpoint by regulating the expression of genes involved in mitochondrial and lipid homeostasis, as well as inflammation and immunity [120]. We have also demonstrated that MNA NB tumors are especially sensitive to BMAL1 activation via genetic and pharmaceutical modalities [120]. The immune system is under circadian control, and BMAL1 and the molecular clock play a central role in regulating the interactions between cancer cells and the TME, including stromal cells, endothelial cells, macrophages, and T cells [121–125]. They also control antigen release and presentation, priming and activation of effector cells, trafficking of immune cells to tumors, and myeloid cell expression of

Clinician's corner

Cancer cells eventually circumvent metabolic inhibition. Thus, targeting multiple arms of cancer metabolism is crucial to prevent therapy resistance. Importantly, normal cells have similar metabolic requirements to tumor cells, and metabolic inhibitors should be considered for their on-target, off-tumor, and immunomodulatory functions. Identifying and employing inhibitors that selectively target tumor metabolic dependencies is essential.

Given the dependency of both tumor and microenvironmental cells on the uptake of exogenous metabolites, altering systemic metabolite levels will have profound effects on both cancer progression and immune cell activity. Importantly, the functional significance of different dietary compositions on neuroblastoma (NB) development and host metabolism remains largely unknown and will require further investigation to best identify tumor-specific metabolic dependencies that avoid impairing antitumor immune cell activity.

Cell-based therapies such as chimeric antigen receptor (CAR)-T cells have had limited success in solid tumors. In NB, anti-GD2 CAR-T cells show some promise, but their antitumor activity could be further enhanced by boosting their intrinsic metabolism or combining them with antitumor metabolic inhibitors.

Although metaiodobenzylguanidine (MIBG) imaging is used clinically to evaluate NB disease burden, many tumors remain MIBG non-avid. Thus, it will be crucial to develop easy-to-implement metabolic platforms for NB imaging that could be transferred to the clinic for a better definition of disease activity and treatment response.

PD-L1 [126,127]. Similarly, immune cells and cancer cells respond differently to immunotherapy as a function of its circadian administration over 24 h [125,128,129]. However, the molecular mechanisms underlying these effects remain to be identified. In this context, MYCN and BMAL1 could have profound effects on the numbers and the phenotype of TILs, and this could have important chronotherapeutic implications. However, how cell-intrinsic clock disruption acts within the TME remains to be determined. Overall, targeting the molecular clock may represent an appealing way to disrupt the MYCN-induced metabolic microenvironment of NB.

Concluding remarks

Therapeutic targeting of MYCN metabolic reprogramming is a novel strategy to improve the outcomes for patients with MYCN-driven tumors. Although this review summarizes individual metabolic pathways, metabolic networking is certainly far more complex because there are interactions between metabolic pathways and crosstalk between tumor genetics/epigenetics, the TME, the microbiota, nutrient availability, and circadian rhythms. Further, the metabolism of micronutrients such as vitamins, minerals, and other cofactors is outside the scope of this review and remains an underexplored field. Because of the shared metabolic requirements of tumor cells and normal proliferating cells such as activated T cells [130], we are challenged to identify essential metabolic nodes that specifically affect tumor fate. Although many drugs targeting essential metabolic nodes have been developed, these drugs have a few major limitations: (i) they often have limited efficacy as single agents and toxicities can arise when used in combination, and (ii) suppression of one metabolic pathway likely induces compensatory responses in another. Future efforts will need to dissect strategies for targeting multiple arms of NB tumor metabolism and to develop combination therapies with FDA-approved DFMO.

Targeting the TME to enhance antitumor activity is a promising therapeutic option for NB. Nevertheless, systematic dissection of the metabolic TME is challenging: (i) tumor cells, immune cells, and stromal cells rely on shared metabolites for both antitumor and protumor functions, making targeting an individual cell type difficult, (ii) as with intratumoral metabolism, targeting one pathway may induce unintended compensation via alternative pathways, and (iii) inter- and intra-tumoral heterogeneity requires both personalized medicine approaches and warrants investigation into targeting of specific spatial niches within an individual TME. Further, our understanding of the TME metabolic landscape through the studies summarized in this review is limited by the current available tools; however, novel technologies are being developed and will be essential for propelling the field forward (Box 2).

Future work will need to consider these complexities and current methodology limitations to investigate mechanisms for tipping the balance of MYCN-driven metabolic competition in favor of antitumor immunity (see Outstanding questions). Further, investigations of novel strategies for patient stratification, via metabolic gene expression or metabolic landscape phenotypes, may reveal subcategories of tumors that would better respond to targeted metabolic therapies. The rewired metabolic landscape of NB is a complex dialogue between tumor cells, TME cells, and the host. Therapeutic targeting of this landscape holds immense potential; however, additional studies will be necessary to develop novel and rationale therapies that account for the complexity of the landscape to best improve NB patient outcomes.

Outstanding Questions

How can researchers better identify essential metabolic hubs to more effectively shut down cancer metabolism? Which metabolic hubs are shared between tumor and microenvironmental cells? What are the effects of targeting these shared metabolic hubs?

Can specific MYCN-induced metabolic circuits be targeted to reactivate the profoundly suppressive microenvironment of high-risk NB? Can this enhance immune-based therapeutic strategies?

Can single-cell metabolomic techniques be employed to better understand how MYCN rewires the metabolic microenvironment of NB or other diseases?

Can CAR-T cells outcompete tumor cells for essential metabolites? Can we generate CAR-T cells that are more metabolically fit to compete in the nutrient-depleted TME or that degrade immunosuppressive metabolites?

How can specific dietary compositions impact NB cell metabolism and its interactions with the TME? What is the role of maternal nutrition in cancer development?

How do host, tissue, and cellular circadian rhythms impact tumor metabolic homeostasis and response to metabolic inhibition? How can we best exploit distinct circadian features for chronotherapeutic approaches?

Acknowledgments

This work was supported by Cancer Prevention and Research Institute of Texas (CPRIT) Baylor College of Medicine Comprehensive Cancer Training Program (RP210027 to A.W.), the National Institutes of Health (NIH; R01CA283369 to E.B.), the Department of Defense (DOD; W81XWH-19-1-0556 to E.B.), and CPRIT (RP240291 to E.B.).

Declaration of interests

The authors declare no competing interests.

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