Review

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Focus: Metabolism

MYC and tumor metabolism: chicken and egg

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Abstract

Transcription factors of the MYC family are deregulated in the majority of all human cancers. Oncogenic levels of MYC reprogram cellular metabolism, a hallmark of cancer development, to sustain the high rate of proliferation of cancer cells. Conversely, cells need to modulate MYC function according to the availability of nutrients, in order to avoid a metabolic collapse. Here, we review recent evidence that the multiple interactions of MYC with cell metabolism are mutual and review mechanisms that control MYC levels and function in response to metabolic stress situations. The main hypothesis we put forward is that regulation of MYC levels is an integral part of the adaptation of cells to nutrient deprivation. Since such mechanisms would be particularly relevant in tumor cells, we propose that—in contrast to growth factor-dependent controls—they are not disrupted during tumorigenesis and that maintaining flexibility of expression is integral to MYC's oncogenic function.

Keywords cancer; metabolic reprogramming; MYC; oncogene; RNAPII
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Introduction

MYC proteins form a small family of closely related oncoproteins, three of which (MYC, N-MYC, L-MYC) have been implicated in the genesis of multiple human tumors (Dang, 2012; Kress *et al*, 2015). Their expression is tightly controlled by growth factor-dependent signals in normal cells and is deregulated and enhanced via multiple mechanisms in tumor cells. A large body of evidence demonstrates unequivocally that enhanced MYC expression is a major driving force of tumorigenesis and that both MYC-driven tumors and tumors driven by other oncogenes, for example, by mutant RAS, continuously depend on elevated MYC levels for growth (Felsher & Bishop, 1999; Shachaf *et al*, 2004; Soucek *et al*, 2008; Annibali *et al*, 2014). Not surprisingly, many biochemical properties appear to be identical among all three proteins, and we will therefore focus on the founding member of the family, MYC, in this review.

For a long time, MYC proteins were considered to be gene-specific transcription factors. This view has been challenged by recent work demonstrating that MYC proteins bind to virtually all active promoters and many enhancers. Consistently, in some situations MYC proteins globally regulate transcription by RNA polymerase II (RNAPII) and also by RNA polymerases I and III (Wolf *et al*, 2015). Exactly whether MYC proteins are oncogenic since they regulate—a large number of—specific target genes or whether they have unique biochemical functions during expression of all genes (e.g., during the release of RNAPII from a promoter-proximal pause position into active elongation) is therefore intensely debated (Dang, 2012; Kress *et al*, 2015; Wolf *et al*, 2015). In this review, we will leave this debate aside and use a relaxed definition of a target gene of MYC as any gene for which relative mRNA levels change in a given experimental situation when MYC levels are altered.

Deregulated expression of MYC promotes proliferation and growth of cells and alters intermediary metabolism to match the enhanced demand for anabolic metabolites (Morrish *et al*, 2009; van Riggelen *et al*, 2010; Dang, 2013; Cunningham *et al*, 2014). This "metabolic reprogramming" is observed both in tissue culture and in transgenic models, in which MYC is expressed from constitutive promoters to mimic the release from its growth factor-dependent control mechanisms (Yuneva *et al*, 2012; Shroff *et al*, 2015). In this review, we describe downstream effects of MYC highlighting the ability of this oncogene to globally rewire cellular metabolism. Notably, effects on cellular metabolism depend both on oncogene activity and on the tissue of tumor origin and on interaction with microenvironment components (Yuneva *et al*, 2012; Davidson *et al*, 2016; Mayers and Vander Heiden, 2017). Metabolic changes induced by deregulated MYC can therefore vary among different tumor types.

As consequence of metabolic changes, deregulated MYC expression can induce metabolic stress: For example, enhanced expression of MYC causes a decrease in cellular ATP levels and activate AMP-activated kinase (AMPK) (Liu *et al*, 2012a; von Eyss *et al*, 2015). MYC-induced metabolic stress sensitizes cells toward apoptosis, in part since AMPK phosphorylates p53 and activates its mitochondrial pro-apoptotic functions (Nieminen *et al*, 2007, 2013). Similarly, loss of mechanisms that restrain MYC activity following hypoxia can result in MYC-driven apoptosis (Brunelle *et al*, 2004). As consequence, cells expressing deregulated MYC can be dependent on a continuous and high supply of nutrients. For example, MYC-driven tumor cells are dependent on a constant supply of glutamine and glutamine deprivation or inhibition of glutaminase can selectively kill cells with enhanced levels of MYC (Yuneva *et al*, 2007; Wise *et al*, 2008; Qing *et al*, 2012; Nieminen *et al*, 2013; Wiese *et al*, 2015). This phenomenon has been termed "glutamine addiction" and provides the rational basis for exploring the therapeutic value of glutaminase inhibitors for MYC-driven tumors. Similarly, rat fibroblast cells overexpressing MYC undergo apoptosis when

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deprived of glucose cells (Shim *et al*, 1998) and MYC-driven lymphoma cells are particularly sensitive to inhibition of lactate export (Doherty *et al*, 2014). An attractive concept is to exploit these dependencies for tumor therapy.

The aim of this review is to summarize the opportunities for targeting the metabolism of MYC-driven tumors for therapy and to identify the critical challenges that need to be addressed for such an approach to be successful. One central argument we put forward is that multiple mechanisms link MYC expression to the metabolic status of tumor cells and that modeling approaches need take to them into account in order not to overestimate the metabolic liabilities caused by enhanced MYC expression.

Oncogenic MYC promotes anabolic reactions: the role of glucose

Cancer cells typically display high glucose uptake, glycolytic metabolism, and lactate production even in the presence of oxygen ("aerobic glycolysis"), a phenomenon termed the Warburg effect (Vander Heiden *et al*, 2009). This seemingly wasteful use of glucose is most likely due to the higher capacity of glycolysis to produce ATP compared to the oxidative phosphorylation; hence, aerobic glycolysis may satisfy the high demand of rapidly growing cells for ATP (Vander Heiden *et al*, 2009; Liberti & Locasale, 2016). Metabolic labeling using ^{13}C-glucose reveals that oncogenic levels of MYC promote high consumption of glucose, as assessed both in Burkitt's lymphoma (Le *et al*, 2012; Murphy *et al*, 2013) and in MYC-driven liver carcinoma (Yuneva *et al*, 2012). MYC exerts its effects on glucose metabolism by increasing the expression of the glucose transporter GLUT1 and by upregulating the expression of glycolytic enzymes, including hexokinase 2 (HK2), phosphofructokinase-M 1 (PFKM1), and enolase 1 (ENO1; Osthus *et al*, 2000; Kim *et al*, 2004). Aerobic glycolysis is also driven by the expression of the M2 isoform of the pyruvate kinase (PKM2), which is expressed in virtually all tumors (Israelsen & Vander Heiden, 2015). MYC enhances expression of PKM2 by promoting the expression of hnRNP splicing factors, as demonstrated in glioma (David *et al*, 2010; Luan *et al*, 2015; Fig 1).

During glycolysis, NAD^+ is reduced to $NADH^+ + H^+$ and cells need to regenerate NAD^+ to maintain the glycolytic flux. This depends on *LDHA*, encoding for lactate dehydrogenase A, which utilizes pyruvate as substrate derived from both the glycolytic and the glutaminolytic pathway and converts it into lactate (Shim *et al*, 1998; Wise *et al*, 2008). Overexpressing MYC enhances *LDHA* expression and typically results in extracellular acidification due to the increased production of lactate (Shim *et al*, 1998; Lewis *et al*, 2000; Fan *et al*, 2010; Maya-Mendoza *et al*, 2015). MYC also promotes the secretion of lactate through the expression of the bidirectional monocarboxylate transporter MCT1 (Doherty *et al*, 2014). Lactate is not simply a waste product of tumors, but an active role of this metabolite in promoting multiple pro-oncogenic functions emerged over time (San-Millan & Brooks, 2016). An indirect mechanism through which MYC promotes the uptake of glucose is via blocking the transcriptional function of MondoA, which in turn results in the inhibition of the thioredoxin-interacting protein (TXNIP), a negative regulator of glycolysis (Shen *et al*, 2015). Intriguingly, deregulated MYC expression also blocks the induction of mRNAs encoding gluconeogenesis enzymes in response to a high-fat diet in liver, arguing that repressive functions of MYC contribute to alterations in cellular metabolism (Riu *et al*, 2003).

The high K_m of PKM2 for phosphoenolpyruvate increases concentrations of glycolytic intermediates and channels them into two biosynthetic pathways (Israelsen & Vander Heiden, 2015), Fig 1. The pentose phosphate pathway (PPP) consists of two branches: the oxidative phase and the non-oxidative phase. The first one uses glucose 6-phosphate to produce both ribose sugars for nucleotide biosynthesis and NADPH for sustaining anabolic reactions; the non-oxidative phase allows for the recycling of glycolytic substrates, like fructose 6-phosphate and glyceraldehyde 3-phosphate, which can be rechanneled into the oxidative arm depending on the cellular metabolic needs (Patra & Hay, 2014). Serum-induced MYC activation in rat fibroblasts increases the flux of glucose toward ribose sugars (Morrish *et al*, 2009). In activated T cells, MYC increases the expression of *G6PDH*, encoding for glucose-6-phosphate dehydrogenase, which catalyzes the first reaction of the oxidative phase, and *TKT*, which encodes for transketolase, an enzyme of the non-oxidative branch (Wang *et al*, 2011).

The serine synthesis pathway (SSP) represents a second important branch of glycolysis, and the activity of the SSP is often upregulated in cancer. Serine-derived glycine fuels the one-carbon metabolism, constituted by two interconnected pathways: the folate and the methionine metabolism. Such reactions are in turn needed for the biosynthesis of macromolecules like purines and glutathione (Yang & Vousden, 2016). The conversion of serine to glycine is catalyzed by the serine hydroxymethyltransferase (SHMT). Both the cytosolic and the mitochondrial isoforms of this enzyme (SHMT1 and SHMT2, respectively) are targets of MYC, as first demonstrated in rat fibroblasts (Nikiforov *et al*, 2002). Increased glycine synthesis resulting from the upregulation of enzyme involved in the SSP is dependent on the oncogenic activity of MYC, as demonstrated in both hepatocellular carcinoma cell lines (Sun *et al*, 2015) and in a MYC-driven liver tumor (Anderton *et al*, 2017).

Glucose also serves as sugar donor for posttranslational modifications of proteins. This occurs through the hexosamine biosynthetic pathway (HBP) in which both glucose and glutamine are metabolized to produce UDP-N-acetylglucosamine (UDP-Glc-NAc). The N-acetylglucosamine is in turn transferred from UDP-Glc-NAc to serine or threonine residues of substrate proteins, a reaction catalyzed by the enzyme O-GlcNAc transferase (OGT). The HBP is hyperactivated in a variety of cancers and O-GlcNAcylated proteins regulate multiple oncogenic processes (Ferrer *et al*, 2016). In addition to promoting the uptake of glucose and glutamine and to channeling these substrates into the HBP (Morrish *et al*, 2009), in breast cancer cells MYC increases the expression of the chaperone protein HSP90, which stabilizes OGT, thus promoting the modification of target proteins (Sodi *et al*, 2015). MYC itself is an OGT target and MYC O-GlcNAcylation on Thr58 increases its stability and competes with the phosphorylation on the same site, which primes MYC for degradation (Itkonen *et al*, 2013).

Oncogenic MYC promotes anabolic reactions: the role of glutamine

Consistent with the increased demand of tumor cells for glutamine, several transporters have been shown to be upregulated in many

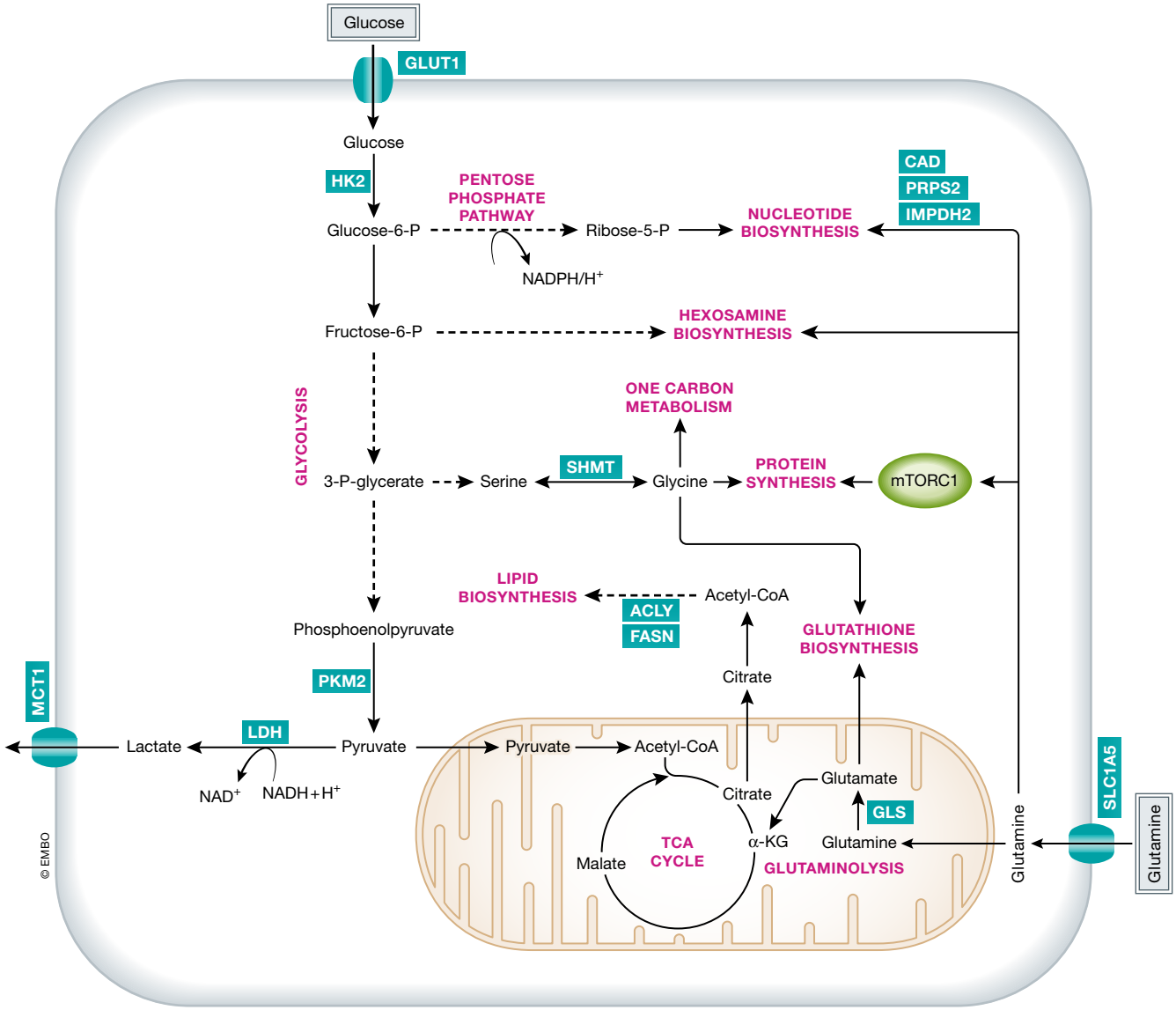

Figure 1. Overview of metabolic pathways promoted by MYC.
MYC increases the uptake and the metabolism of glucose and glutamine, the major nutrients of cancer cells, by upregulating the expression of genes encoding for membrane transporters and metabolic enzymes (shown in turquoise; see text). MYC globally promotes anabolic reactions (indicated in magenta) in order to sustain the biosynthetic needs of proliferating cells. Full arrows show direct reactions, and dashed ones indicate the presence of metabolic intermediates. GLUT1: glucose transporter 1; HK2: hexokinase 2; PKM2: pyruvate kinase M2; LDH: lactate dehydrogenase; MCT1: monocarboxylate transporter 1; SHMT: serine hydroxymethyltransferase; ACLY: ATP citrate lyase; FASN: fatty acid synthase; CAD: carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase; PRPS2: phosphoribosyl pyrophosphate synthetase 2; IMPDH2: inosine-5′-monophosphate dehydrogenase 2; SLC1A5: solute carrier 1A5; GLS: glutaminase; TCA: tricarboxylic acid; glucose-6-P: glucose 6-phosphate; fructose-6-P: fructose 6-phosphate; 3-P-glycerate: 3-phosphoglycerate; ribose-5-p: ribose 5-phosphate; α-KG: α-ketoglutarate; NADPH: nicotinamide adenine dinucleotide phosphate; NADH: nicotinamide adenine dinucleotide.

types of cancer (Bhutia & Ganapathy, 2016). SLC1A5 (ASCT2) and SLC38A5 represent the best characterized glutamine transporters; both of them are upregulated by MYC in glioma cell lines (Wise *et al*, 2008), and a positive correlation between N-MYC amplification and *ASCT2* expression has been observed in neuroblastoma cells (Qing *et al*, 2012; Ren *et al*, 2015). Such membrane proteins transport also additional amino acids, like serine and alanine (Bhutia & Ganapathy, 2016), that can be channeled into biosynthetic pathways.

A major function of glutamine in cancer is to enter the TCA cycle in the form of α-ketoglutarate (α-KG), through a process termed glutaminolysis. MYC promotes the glutaminolytic pathway by upregulating the expression of *GLS*, encoding for the enzyme glutaminase, which catalyzes the conversion of glutamine to glutamate (Wise *et al*, 2008; Gao *et al*, 2009). *GLS* expression is upregulated by MYC both directly or indirectly, through MYC-mediated inhibition of miR-23 expression, which in turn suppresses *GLS* translation (Gao *et al*, 2009). Another study demonstrated that MYC

controls GLS levels in an mTOR-dependent way (Csibi *et al*, 2014). *In vivo* studies confirmed that MYC impacts on the metabolism of glutamine mainly by enhancing the expression of *GLS1*, as demonstrated in both liver and renal carcinoma models (Yuneva *et al*, 2012; Shroff *et al*, 2015; Xiang *et al*, 2015). Glutamine-derived glutamate enters the TCA cycle as α-KG either by direct deamination catalyzed by glutamate dehydrogenase (GLUD) or through the action of transaminases, which catalyze the transfer of the amino group to oxaloacetate (glutamate oxaloacetate aminotransferase or GOT) or pyruvate (glutamate pyruvate aminotransferase or GPT) with the simultaneous production of the non-essential amino acids aspartate and alanine, respectively. Increased levels of both aspartate and alanine have been detected in MYC-driven renal carcinoma (Shroff *et al*, 2015) and in pre-tumorigenic liver expressing high MYC levels (Hu *et al*, 2011). Decreased levels of GPT and GOT have been observed in MYC-dependent osteosarcoma cells upon suppression of MYC expression (Anso *et al*, 2013). Dependency of cancer cells on either GLUD or transaminases activity has been correlated with high levels of MYC (Yang *et al*, 2009; Qing *et al*, 2012; Korangath *et al*, 2015).

The TCA cycle is used as a source of multiple precursors, which are diverted into biosynthetic pathways in order to sustain the requirements of highly proliferative cells. Hence, the anaplerotic function of glutamine, that is, the replenishment of TCA cycle intermediates, is important for supporting anabolic reactions (DeBerardinis *et al*, 2008). For example, *de novo* synthesis of fatty acids uses acetyl-CoA as a substrate and enzymes belonging to this pathway are often upregulated in cancer. Acetyl-CoA can be derived from both glucose and glutamine, through the cytosolic conversion of TCA-produced citrate by ATP citrate lyase (ACLY). In the cytosol, acetyl-CoA is converted by acetyl-CoA carboxylase (ACC) to malonyl-CoA, which is in turn used for the biosynthesis of palmitate by fatty acid synthase (FASN). Several studies suggest that MYC may promote the synthesis of fatty acids, although deeper mechanistic investigations will be needed to corroborate such evidences. MYC promotes fatty acid biosynthesis in rat fibroblasts through increasing the expression of all three enzymes (Morrish *et al*, 2009, 2010; Edmunds *et al*, 2014). Metabolic analysis performed on a combination of engineered cells, *in vivo* models, and human tumor samples showed that deregulated MYC in prostate cancer specifically enhances lipid metabolism (Priolo *et al*, 2014). Specific lipid production associated with the oncogenic activity of MYC has been observed also in lymphomas. Increased levels of phosphatidylglycerol, a precursor of cardiolipin, are found on the outer mitochondrial membrane, in MYC-driven lymphoma tumor models compared to normal thymus (Eberlin *et al*, 2014). The same lipid is also increased in *in vivo* models of MYC-driven hepatocellular carcinoma and renal cell carcinoma (Perry *et al*, 2013; Shroff *et al*, 2015).

Glutamine-derived glutamate can also be used as substrate for proline synthesis. Although the conversion between the two amino acids is reversible, MYC preferentially suppresses the proline to glutamate production in favor of the opposite reaction, as demonstrated in Burkitt's lymphoma and prostate cancer (Liu *et al*, 2012b, 2015). The first effect is due to MYC-induced expression of miR-23b*, which in turn posttranscriptionally suppresses the translation of *PRODH*, encoding for the enzyme proline oxidase (or proline dehydrogenase). Interestingly, miR-23b* is produced from the same transcript of miR-23, which suppresses *GLS* translation; thus, oncogenic MYC exerts a coordinated effect in promoting the catabolism of glutamine, rather than its synthesis, as demonstrated also by the inverse correlation between MYC and GS levels (Liu *et al*, 2012b, 2015). At the same time, MYC enhances the expression of enzymes involved in the biosynthesis of proline, namely P5C synthase (P5CS) and P5C reductase (PYCR) (Liu *et al*, 2012b, 2015). Blocking proline biosynthesis inhibited cell growth in a way independent from exogenous proline supplementation. The synthesis of proline yields $NAD(P)^+$ through the conversion of 1-pyrroline-5-carboxylate (P5C) to proline catalyzed by PYRC, which is in turn required for sustaining both glycolysis and the oxidative arm of the PPP (Liu *et al*, 2012b, 2015).

Importantly, *in vivo* studies document a remarkable flexibility in the metabolic features of MYC-driven tumors. For example, MYC-induced liver tumors consume glutamine, while lung tumors produce it. This difference is mainly due to difference in expression of glutamine synthetase (GS), which catalyzes the cytosolic production of glutamine from glutamate and ammonia, and is expressed in lung, rather than in liver tumors (Yuneva *et al*, 2012). Expression of GS expression makes cancer cells independent of the external glutamine supply (Kung *et al*, 2011). Interestingly, oncogenic MYC can also drive and increase in GS expression. Bott *et al* showed that MYC induces the expression of thymine DNA glycosylase in breast cancer cell lines. This in turn promotes the demethylation of the GS promoter, thus enhancing *GS* expression. Increased glutamine production supports cell proliferation through increased nucleotide biosynthesis (Bott *et al*, 2015). Thus, it is likely that MYC can differentially promote the synthesis of glutamine or its metabolism through the TCA cycle, depending on the context and the metabolic needs of the tumor. Similarly, while MYC often promotes the biosynthesis of lipids, MYC overexpression in triple-negative breast cancer correlates with an increased dependency on mitochondrial fatty acid oxidation, whose inhibition has been proposed as a possible therapeutic approach (Camarda *et al*, 2016). The divergence of effects mediated by oncogenic MYC highlights the importance of *in vivo* studies to better understand the metabolic requirements of tumors, since both the interaction with the tumor microenvironment and an intact organ structure affect metabolic dependencies. This has been clearly demonstrated using K-RAS-driven lung tumor cells, which are glutamine dependent *in vitro*, but not *in vivo* (Davidson *et al*, 2016).

Oncogenic MYC enhances the biosynthesis of nucleotides

Increased nucleotide biosynthesis is required to sustain the enhanced transcription and replication driven by MYC. The biosynthesis of purine and pyrimidine nucleotides requires substrates whose metabolism is controlled by MYC, for example, the amino acids aspartate and glycine, in addition to glutamine, while glucose metabolism provides both ATP and the ribose moiety necessary for the biosynthesis of phosphoribosylpyrophosphate (PRPP), through the PPP. MYC directs the channeling of these substrates toward the biosynthesis of nucleotides and promotes their usage by enhancing the expression of enzymes involved in virtually all steps of these pathways (Liu *et al*, 2008; Mannava *et al*, 2008). In addition, MYC promotes the reduction in ribonucleotides to deoxynucleotides through the upregulation of *RRM2*, encoding for the small subunit of the enzymatic complex ribonucleotide reductase (Mannava *et al*, 2008). Thus, high MYC levels result in an increase in both ribo- and

deoxyribonucleotide levels, which is observed in multiple cellular systems (Bester *et al*, 2011; Cunningham *et al*, 2014). Phosphoribosylpyrophosphate is a key substrate for both the purine and the pyrimidine biosynthesis. In the first case, the purine ring is assembled around a PRPP molecule, while in the second pathway PRPP is attached to the synthetized pyrimidine ring. Oncogenic MYC upregulates the expression of *PRPS2*, encoding for the enzyme, which catalyzes PRPP synthesis from ribose 5-phosphate and ATP (Mannava *et al*, 2008; Cunningham *et al*, 2014). Cunningham *et al* showed that MYC exerts a translational control on PRPS2, mediated by pyrimidine-rich translational elements present in the 5′-UTR of PRPS2 and not of its related isoform PRPS1. Effects on the translation of specific mRNA are achieved by MYC through the upregulation of the eukaryotic translation initiation factor 4E (eIF4E), the limiting factor of the initiation complex eIF4F (Ruggero *et al*, 2004). This mechanism may coordinate protein and nucleotide biosynthesis in an oncogenic setting. Interestingly, PRPS2 is dispensable for the development of normal B cell but its inhibition is synthetic lethal with MYC overexpressing cells, possibly by limiting the amount of nucleotides available for assembly of ribosomes and the consequent protein synthesis (Cunningham *et al*, 2014).

Polyamine biosynthesis

A possible use of glutamine is the biosynthesis of polyamines (putrescine, spermidine, and spermine). Polyamine are polycations able to interact with negatively charged molecules such as nucleic acids and protein. They have multiple functions in the regulation of cell growth, modulation of histone acetylation levels, and intracellular signaling (Minois *et al*, 2011). Increased polyamine levels are associated with a variety of cancers, and therapeutic approaches targeting the metabolism of polyamine have been developed (Murray-Stewart *et al*, 2016). The biosynthesis of polyamine begins from ornithine and requires decarboxylated S-adenosyl-methionine produced from methionine for the subsequent steps. Although glutamine-derived glutamate or proline represent alternative substrates for the production of ornithine, compared to the mostly used arginine, in activated T lymphocytes MYC has been observed to drive the biosynthesis of polyamines through the increased expression of enzymes, which mediate both the conversion of glutamate and proline to ornithine [aldehyde dehydrogenase 18 family member A1 (Aldh18a1), proline dehydrogenase (Prodh) and ornithine aminotransferase (OAT)] and the biosynthesis of polyamines [ornithine decarboxylase (ODC), spermine synthase (SRM) and spermidine synthase (SMS)] (Bello-Fernandez *et al*, 1993; Trubiani *et al*, 1999; Nilsson *et al*, 2005; Wang *et al*, 2011; Funakoshi-Tago *et al*, 2013; Ruiz-Perez *et al*, 2015). The coordinated expression of genes encoding polyamine biosynthetic enzymes is also observed in a mouse model of MYC-driven B-cell lymphoma (E_{μ}-*Myc*). Increased expression of ODC, the rate-limiting enzyme in the polyamine biosynthesis, is a critical mediator of MYC-induced tumorigenesis, since genetic or drug-mediated inhibition of ODC results in an inability of MYC to downregulate the cyclin-dependent kinase inhibitors p21 and p27, thus impairing the progression through the cell cycle. Intriguingly, the activity of ODC and its metabolic products appear to be necessary for tumor development rather than maintenance, before the occurrence of additional genetic events like the loss of the tumor suppressor (Nilsson *et al*, 2005).

Metabolic controls of MYC activity

MYC is both upstream and downstream of mTORC1

Expression of MYC is downstream of multiple control mechanisms that are regulated by nutrient levels and respond to metabolic stress (Fig 2). A paradigm example is the mTOR pathway, the nutrient-sensing pathway in mammalian cells. The activity of mTORC1, a

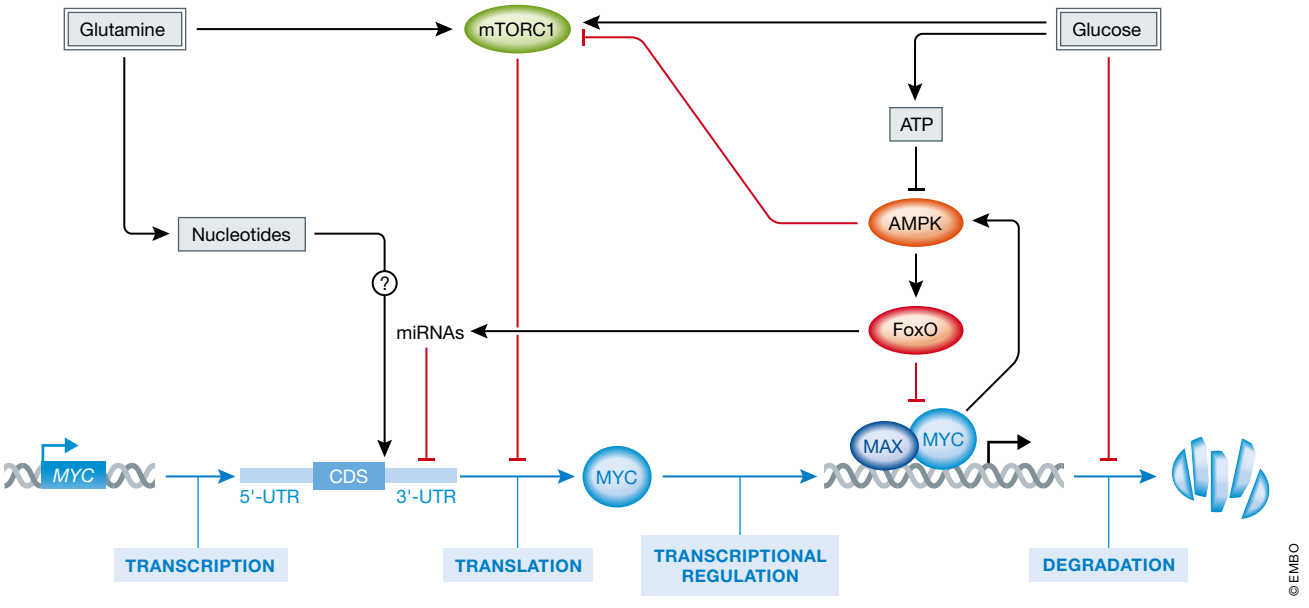

Figure 2. Metabolic control of MYC protein levels.
Sufficient levels of nutrients like glucose, glutamine, and their derived metabolites maintain high levels of MYC. The graph summarizes mechanisms discussed in the text.

central kinase in the pathway, depends on both the availability of nutrients and on the cellular energetic status (Gonzalez & Hall, 2017). Glutamine positively controls mTORC1 activity. Glutamine activates mTORC1 by either promoting the uptake of leucine, which in turn directly activates mTORC1 (Nicklin *et al*, 2009) or through the production of α-KG via the glutaminolytic pathway (Duran *et al*, 2012). The first mechanism is promoted by the amino acid transporter LAT1, which exchanges intracellular glutamine with extracellular leucine. Both MYC and N-MYC enhance the expression of *SLC7A5*, which encodes for a subunit of LAT1 (Gao *et al*, 2009; Hayashi *et al*, 2012; Qing *et al*, 2012); thus, MYC collectively promotes both the uptake of glutamine and its exchange with leucine. Leucine is also an activator of the enzyme glutamate dehydrogenase (GDH), which converts glutamate to α-KG (Couee & Tipton, 1989). Thus, by enhancing the uptake of leucine, MYC potentially contributes to stimulate the glutaminolytic pathway. In addition to enhancing the uptake of nutrients, MYC sustains the function of mTORC1 also by driving the synthesis of macromolecules, like translation factors and ribosomal components, necessary for protein biosynthesis (Lin *et al*, 2008; van Riggelen *et al*, 2010; Pourdehnad *et al*, 2013).

Conversely, mTORC1 controls the translation of MYC (West *et al*, 1998; Shahbazian *et al*, 2010; Csibi *et al*, 2014; Leu *et al*, 2016). An exemplar mechanism indicating the tight connection between MYC and mTORC1 activity is provided by the work of Csibi *et al* (2014), who observed that the mTORC1 target S6K1 promotes MYC translation by acting on its 5′-UTR and that MYC, in turn, increases the protein levels of GLS, likely in a posttranscriptional way since no changes in the *GLS* mRNA levels were observed. MYC and mTORC1 coordinate multiple processes that promote cellular growth and proliferation and overlapping metabolic functions between these two nodes have been demonstrated, for example, both drive the expression of nucleotide biosynthetic enzymes (Liu *et al*, 2008; Mannava *et al*, 2008; Ben-Sahra *et al*, 2013, 2016).

FoxO proteins antagonize MYC function

A second example of the interplay between MYC expression and metabolism is provided by the forkhead box O (FoxO) family of transcription factors. FoxO proteins are activated in response to various kinds of stress, including metabolic and oxidative stress (Eijkelenboom & Burgering, 2013). For example, activation of AMPK-activated protein kinase (AMPK) stimulates FoxO activity (Greer *et al*, 2007). Following increase in the AMP/ATP ratio, indicator of changes in the cellular energetic status, activated AMPK phosphorylates FoxO3 on multiple sites and promotes its transcriptional activity. Such effect supports cell survival under stress conditions due to the FoxO3-mediated activation of genes involved in stress resistance, for example, by promoting the metabolism of alternative sources in absence of glucose, maintaining intracellular energetic homeostasis through autophagy or reducing oxidative stress (Greer *et al*, 2007; Chiacchiera & Simone, 2009; Li *et al*, 2009).

FoxO proteins antagonize activation of multiple target genes by MYC via several different mechanisms (Bouchard *et al*, 2004). On multiple MYC target genes, non-phosphorylated FoxO blocks the loading of RNAPII on the promoter, thereby blunting the ability of MYC to promote transcriptional elongation by RNAPII (Bouchard *et al*, 2004). In hypoxia, HIF-1-α activates FoxO3A, which displaces MYC from the promoter of mitochondrial genes (e.g., *MRPL12* (mitochondrial ribosomal protein L12), *ACO2* (aconitase 2), *LARS2* (mitochondrial leucyl-tRNA synthetase), and *OXNAD1* (oxidoreductase NAD-binding domain containing 1)) (Jensen *et al*, 2011). Thus, by disabling the MYC-mediated induction of mitochondrial genes, FoxO3A promotes the shift toward a glycolytic metabolism. MYC function can also be antagonized by FoxO3A through the increased expression of Mad/Mxi family proteins, particularly Mxi1-SRα that binds to MAX and prevent the formation of the MYC/MAX heterodimers (Delpuech *et al*, 2007). Activation of FoxO3A can also destabilize MYC and induce its proteasomal degradation. The decrease in MYC protein levels diminishes the production of reactive oxygen species (ROS) by reducing mitochondrial activity, thereby preventing harmful effects associated with increased ROS levels such as genomic instability due to ROS-induced DNA damage. (Ferber *et al*, 2012). The 3′-UTR of *MYC* is also targeted by FoxO-dependent stress-responsive circuits. FoxO3A can limit MYC levels by inducing the expression of miR-34b/c, which bind to the 3′-UTR of MYC, thereby inhibiting its translation (Kress *et al*, 2011). Different stimuli can mediate the induction of specific miRNAs through FoxOs. In glioblastomas, for example, FoxOs induce both miR-34c and miR-145 to repress MYC (Masui *et al*, 2013).

Repression of MYC function by FoxOs is antagonized by mTORC2, which promotes FoxOs acetylation, and by FoxOs phosphorylation following PI3K-AKT activation (Masui *et al*, 2013). Both pathways are physiologically activated by growth factors stimulation and hyperactivated in a cancer context (Fruman & Rommel, 2014). Consistently, the ability of FoxO proteins to suppress MYC function in response to metabolic stress may be compromised in tumors. For example, regulation of MYC via the 3′-UTR is disrupted during colon cancer progression, through the silencing of miR-34b/c expression (Kress *et al*, 2011). Similarly, the FoxO/Mxi1-SRα/miR-145 axis, which antagonizes MYC function and decreases its levels, operates *in vivo* in *Tsc1* knockout (i.e., mTORC1 activation) polycystic kidneys, but is lost in *Tsc1* knockout kidney tumors (Gan *et al*, 2010). Intriguingly, disruption of the FoxO/MYC regulatory circuit can have both positive and negative effects on tumor growth. For example, FoxO3A knockdown impaired the growth of tumors in a xenograft model, likely because they maintain the mitochondrial function under hypoxia (Jensen *et al*, 2011).

Regulation of MYC levels and function by glucose

Several mechanisms regulate both MYC levels and function in response to glucose availability. Increased degradation of MYC following glucose starvation and oxygen deprivation maintains colorectal cancer cells viable, although metabolically inactive (Okuyama *et al*, 2010; Wong *et al*, 2013). MYC is O-glcNAcylated and stabilized by this posttranslational modification (Itkonen *et al*, 2013; Sodi *et al*, 2015). Following glucose deprivation, the decreased OGT activity and consequent increased MYC degradation allow cell survival in hepatocarcinoma cells (Buren *et al*, 2016). Intriguingly, glucose deprivation not only regulates MYC levels via proteasomal degradation, but also induces calpain-mediated proteolysis of MYC, which results in the formation of a truncated protein localized in the cytosol ("MYC-nick"; Conacci-Sorrell *et al*, 2014). MYC-nick comprises the N-terminal region of MYC and has transcription-independent functions since it lacks the nuclear localization signal and the DNA binding domain (Conacci-Sorrell *et al*, 2010). Strikingly, MYC-nick protects cells from stress-induced cell

death, since it promotes the acetylation of cytosolic proteins, involved, for example, in sustaining autophagy, via the same domain that recruits histone acetylases in the nucleus (Conacci-Sorrell *et al*, 2014).

MYC is part of an extended network comprising MAX, MXD, MLX, and Mondo proteins. Mondo transcription factors (MondoA and chREBP) represent the nutrient-sensing branch of this network because their activity is directly stimulated by the glycolytic metabolite glucose 6-phosphate (Kaadige *et al*, 2010). Reduced MYC protein levels following glucose starvation may alter the existing balance between each component of the network. Since MYC drives the uptake of glucose by competing with MondoA and downregulating TXNIP expression (Shen *et al*, 2015), one could speculate that in absence of glucose the increased degradation of MYC could positively regulate MondoA and TXNIP function, potentially contributing to maintain the cells in a metabolically inactive state.

Glucose starvation does not uniformly lead to MYC downregulation (Wu *et al*, 2015). Under this condition, upregulation of MYC protein levels occurs in hepatocellular carcinoma cells and supports the synthesis of glutathione through the upregulation of the serine synthesis pathway. Increased SSP activity, in turn, enables cell survival by maintaining the cellular redox balance (Sun *et al*, 2015). Glucose deprivation can also induce the unfolded protein response (UPR) since it interferes with protein glycosylation (Xu *et al*, 2005). Induction of UPR in turn increases MYC levels in multiple myeloma cells via an internal ribosomal entry site (IRES) that is present in the 5′-UTR of the *MYC* mRNA (Shi *et al*, 2016). An increase in MYC levels could therefore be responsible for driving apoptosis under these conditions.

MYC and glutamine: safe or addicted?

The role of glutamine levels as possible upstream regulator of MYC has received less attention. In colorectal cancer cells, translation of MYC is controlled by glutamine via a sequence element in the 3′-UTR of the *MYC* mRNA (Dejure *et al*, 2017). Surprisingly, this regulatory sequence does not respond to TCA cycle intermediates, but to intracellular levels of glutamine-derived adenosine nucleotides. The decrease in MYC levels following glutamine starvation and the consequent reduction in nucleotide levels reduces transcription by RNAPII. When MYC is ectopically expressed in glutamine-starved cells, RNAPII stalls during transcription on hundreds of genes and this correlates with formation of R-loops, which can cause DNA damage and genomic instability (Dejure *et al*, 2017). Notably, cells undergo apoptosis under these conditions in the absence of a MYC-dependent induction of pro-apoptotic genes, suggesting that stalling of RNAPII and R-loop formation are pro-apoptotic events. In this sense, coupling of MYC expression to metabolic signals may be critical for cells to maintain genomic stability.

The precise pathway by which adenosine levels regulate MYC translation has not been resolved. The *MYC* 3′-UTR is the target of multiple miRNAs, which inhibit the translation of an mRNA. Glutamine starvation can induce the phosphorylation of p53 on Ser15, which results in the stabilization of p53. This is required for allowing cell survival under stress conditions (Reid *et al*, 2013). p53, in turn, can trigger the activation of stress-responsive pathways, which can potentially target the 3′-UTR of *MYC* (Sachdeva *et al*, 2009; Cannell *et al*, 2010; Lezina *et al*, 2013). Regulation of RNA-binding proteins (RBPs) targeting the 3′-UTR of *MYC* by cellular metabolism has been also extensively characterized (Barreau *et al*, 2005). For example, a cleavage product of the human antigen R (HuR) (HuR-CP1) associates with *MYC* 3′-UTR, blocking its translation after prolonged hypoxia (Talwar *et al*, 2011). HuR also regulates *MYC* stability in presence of polyamine, whose synthesis is also dependent on glutamine (Liu *et al*, 2009). Finally, multiple metabolic enzymes, for example, glycolytic enzymes, moonlight as RNA-binding proteins via their nucleotide-binding motifs (Castello *et al*,

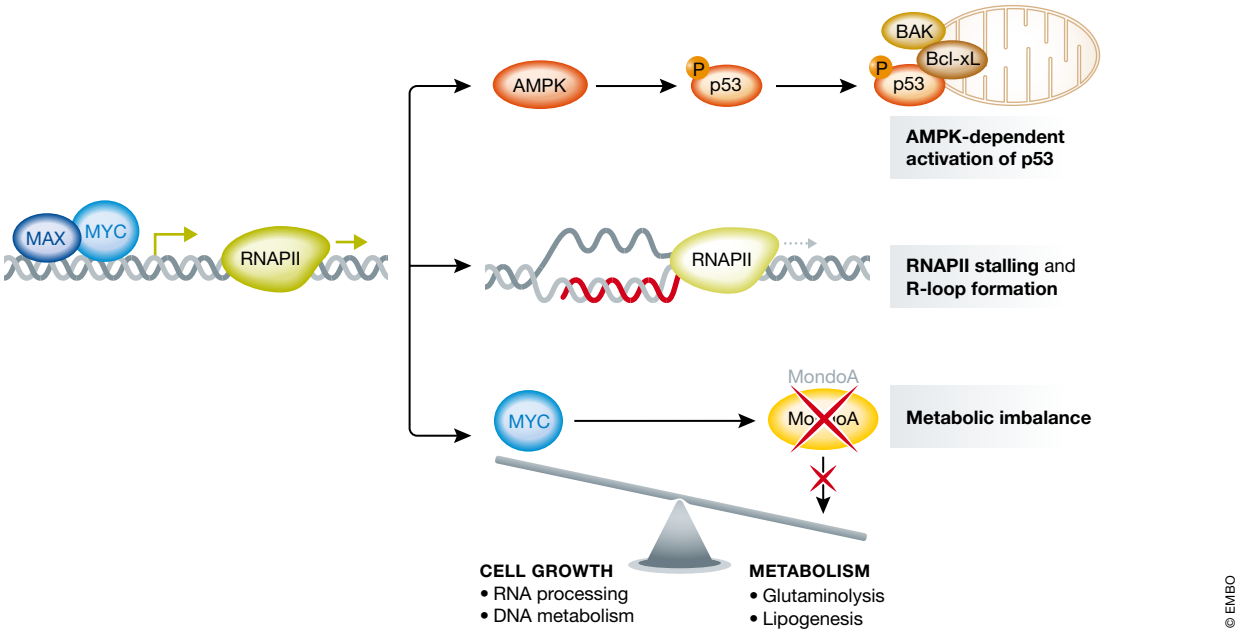

Figure 3. Metabolic adaptation through the regulation of MYC levels.
Proposed mechanisms by which deregulated MYC causes metabolic stress and apoptosis. See text for details.

2012, 2015); hence, the regulation of *MYC* translation may be directly coupled to the metabolic status of cells.

Restraining MYC can prevent apoptosis and metabolic stress

These mechanisms enable cells to maintain cell viability under metabolically unfavorable conditions, through the regulation of MYC levels. On the other side, multiple studies have linked deregulated MYC activity to the induction of cell death as a form of intrinsic tumor suppression (Lowe *et al*, 2004). At least in some instances, MYC-driven apoptosis can be linked to sustained anabolic reaction following nutrient depletion. One of the best characterized examples is provided by the MYC-mediated glutamine addiction, according to which cells expressing high levels of MYC are sensitive to glutamine deprivation or glutaminase inhibition (Yuneva *et al*, 2007; Wise *et al*, 2008; Qing *et al*, 2012; Nieminen *et al*, 2013; Wiese *et al*, 2015). Similarly, rat fibroblast cells overexpressing MYC undergo apoptosis when deprived of glucose (Shim *et al*, 1998) and MYC-driven lymphoma cells are particularly sensitive to inhibition of lactate export (Doherty *et al*, 2014). Since such studies made use of *MYC* transgenes, one possibility to explain opposing observation is the fact that the absence of UTR elements does not allow posttranscriptional mechanisms of regulation to take place. Several mechanisms by which deregulated MYC levels induce metabolism-driven apoptosis have been identified (Fig 3).

- Enhanced expression of MYC causes a decrease in cellular ATP levels and activates AMP-activated kinase (AMPK) (Liu *et al*, 2012a; von Eyss *et al*, 2015). MYC-induced metabolic stress can sensitize cells toward apoptosis, in part since AMPK phosphorylates p53 and activates its mitochondrial pro-apoptotic functions (Nieminen *et al*, 2007, 2013).
- Deregulated MYC can cause transcriptional elongation in the absence of sufficient nucleotides and induce stalling of RNA polymerases and R-loop formation (Dejure *et al*, 2017).
- Deregulated MYC can cause a catastrophic metabolic imbalance. MYC is part of extended network of helix-loop-helix transcription factors and its effects on metabolism are counterbalances by a nutrient-sensing member of the network, MondoA. Reduced MondoA levels are tolerated in normal cells; however, deregulated of MYC renders cells dependent on MondoA, since MondoA counterbalances MYC effects on glutaminolysis and lipid biosynthesis. In the absence of MondoA, cells undergo apoptosis, which can be rescued by fatty acid (oleic acid) supplementation (Carroll *et al*, 2015).

Conclusions

While growth factor-dependent controls of MYC expression often act via the *MYC* promoter, the expression of MYC is controlled mainly by posttranscriptional mechanisms in response to nutrient supply and metabolic stress. There is little evidence that metabolic controls of *MYC* expression and function are disrupted in tumor cells. To the contrary, such controls are likely to be under strong positive selective pressure during tumorigenesis to ensure cell survival under stressed conditions. Currently used transgenic models often bypass these controls, since they use transgenes that express the *MYC* coding sequence only and/or employ stable alleles of *MYC*. It is likely that—by bypassing metabolic controls of MYC expression—current tumor modeling strategies overestimate the metabolic liabilities of MYC-driven tumors.

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