Coordination of Nutrient Availability and Utilization by MAX- and MLX-Centered Transcription Networks

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Cell growth and division require the biosynthesis of macromolecule components and cofactors (e.g., nucleotides, lipids, amino acids, and nicotinamide adenine dinucleotide phosphate [NADPH]). Normally, macromolecular biosynthesis is under tight regulatory control, yet these anabolic pathways are often dysregulated in cancer. The resulting metabolic reprogramming of cancer cells is thought to support their high rates of growth and division. The mechanisms that underlie the metabolic changes in cancer are at least partially understood, providing a rationale for their targeting with known or novel therapeutics. This review is focused on how cells sense and respond transcriptionally to essential nutrients, including glucose and glutamine, and how MAX- and MLX-centered transcription networks contribute to metabolic homeostasis in normal and neoplastic cells.

MYC, also known as c-Myc, is a member of the basic helix-loop-helix leucine zipper (bHLHZip) family of transcription factors. MYC typically functions as a heterocomplex with the MAX bHLHZip protein to bind CANNTG sites that typically lie within a few kilobases of the transcriptional start site of a regulated gene. MYC, as discussed elsewhere in this volume, has pleiotropic functions in cell physiology that stem from its regulation of 10%–15% of the RNA polymerase II-dependent transcriptome, regulation of polymerase I and III transcription and its positive regulation of DNA replication (Eilers and Eisenman 2008). With this broad spectrum of functional targets, it is not surprising that MYC dysregulation is

implicated in up to 70% of human malignancy (Dang 2012b). No single, or even small collection, of MYC target(s) seems to be sufficient for its function as an oncogene. This finding is underscored by two recent reports suggesting that MYC functions as an “amplifier” of gene expression (Lin et al. 2012; Nie et al. 2012). The recent discovery that bromodomain inhibitors JQ1 and IBET-151 result in potent MYC down-regulation raises the possibility that the therapeutic targeting of MYC-dependent cancers may soon be possible (Filippakopoulos et al. 2010; Delmore et al. 2011; Mertz et al. 2011).

One class of MYC targets of particular interest regulate cell metabolism, specifically glycolysis and glutaminolysis (Dang et al. 2009;

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Dang 2012a). A seminal paper from the Dang laboratory in 1997 showed that lactate dehydrogenase is a MYC target and is required for MYC-dependent glycolysis (Shim et al. 1997). It is now known that MYC regulates the expression of virtually every gene encoding an enzyme of the glycolytic pathway. More recent papers show that MYC directly or indirectly, through the regulation of microRNAs, controls the expression of genes required for glutamine uptake or glutamine utilization (Dang 2012b). Glycolysis and glutaminolysis fuel much of macromolecular biosynthesis, establishing MYC as an important regulator of the anabolic processes required for cell growth. Further, targeting MYC-dependent changes in cancer metabolism may be a new and fruitful avenue for the development of new therapeutics. A number of excellent recent review articles highlight dysregulated metabolic pathways in cancer more broadly (Deberardinis et al. 2008; Vander Heiden et al. 2009; Dang 2012a; Ward and Thompson 2012).

Our group identified and has studied the transcription factor MLX interacting protein (MLXIP), also known as MondoA, for several years. MondoA is also a bHLHZip factor and like MYC, MondoA has important functions in cell metabolism. Rather than controlling nutrient utilization directly like MYC, MondoA appears to function primarily in how cells sense, respond to, and coordinate the utilization of glucose and glutamine (Sans et al. 2006; Stoltzman et al. 2008, 2011; Kaadige et al. 2009; Peterson et al. 2010; Peterson and Ayer 2011; Havula and Hietakangas 2012). At present, our understanding of the functional interactions between MYC and MondoA is limited. However, current data suggest that MYC dysregulation and inactivation of MondoA are important drivers of metabolic reprogramming in cancer.

MLXIP/MondoA BASICS

As illustrated in Figure 1, MondoA interacts with another bHLHZip protein called MLX (MAX-like-protein X) and MondoA:MLX heterodimers, following regulated nuclear entry, are capable of binding promoters of target genes containing carbohydrate response ele-

ment (ChoRE) binding sequences, two E-boxes separated by five nucleotides, and regulating their expression (Kaadige et al. 2010; Sloan and Ayer 2010; Peterson and Ayer 2011). At 919 amino acids, MLXIP is among the largest bHLHZip proteins. MLX, as the name implies, is more MAX-like in size, with three isoforms, α , β , and γ , encoded by 214, 244, and 298 amino acids open reading frames, respectively. Compared with MAX, MLX apparently has a more restricted set of protein partnerships. In addition to MondoA, MLX it is known to interact with the MondoA paralog MLX interacting protein-like (MLXIPL), also known as MondoB or carbohydrate response element binding protein (ChREBP), as well as MXD1, MXD4, and MNT (McFerrin and Atchley 2011). The shared binding of MAX and MLX for MXD1, MXD4, and MNT provides a physical connection and the opportunity for functional cross talk between MAX-centered and MLX-centered transcriptional networks (Fig. 1). It is worth noting that the last hydrophobic residue of the leucine zipper of MLX, which mediates heterotypic interactions between bHLHZip proteins, is a tyrosine. This terminal tyrosine is only found in the leucine zipper of MLX family members and is universally conserved across metazoans (McFerrin and Atchley 2011). It is possible that MLX has expanded alternative protein partnerships under signaling conditions that result in phosphorylation of this tyrosine residue.

In addition to the bHLHZip domain, MondoA has a number of other conserved regions of functional significance. MondoA and MLX interact through their bHLHZip and dimerization and cytoplasmic localization domain (DCD). Like MAX, MLX appears to lack a transactivation domain (TAD); however, MondoA has a potent TAD localized in the middle third of its open reading frame (Billin et al. 2000; Billin and Ayer 2006). The amino terminus of the Mondo family (MondoA and ChREBP) has five regions that we have called Mondo conserved regions (MCR). The MCRs are highly conserved across metazoans and are easily identifiable in lower eukaryotes, suggesting an important and ancestral function (Fig. 1) (Kaadige et al. 2010; McFerrin and Atchley

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Figure 1. MAX- and MLX-centered transcriptional network. MAX binding proteins form one node of this transcriptional network, whereas MLX binding proteins form the second node. Competition for binding of MXD1, MXD4, and MNT provide one potential mechanism for interaction between the two nodes of the transcriptional network. MLX isoforms display distinct subcellular localization suggesting that complex switching may occur to facilitate nuclear accumulation and transcriptional regulation. Domain structure of MLXIP including the Mondo conserved regions (MCR), the transcriptional activation domain (TAD), the basic helix-loop-helix leucine zipper (bHLHZip), and the dimerization and cytoplasmic localization domain (DCD). Mutations introduced into individual MCR and bHLHZip domains lead to effects on subcellular localization and transcriptional activity. 100 aa, 100 amino acids.

2011). As described below, the nuclear localization and transcriptional activity of MondoA:MLX complexes is controlled by glucose-6-phosphate (G6P) and we have suggested that G6P interacts directly with the MCRs to control the nuclear activity of MondoA:MLX complexes by an allosteric mechanism (Stoltzman et al. 2008; Peterson et al. 2010). Consistent with this, a recent in-depth phylogenetic study of MondoA's amino terminus proposed the existence of MCR6. This additional domain was suggested based on its similarity to G6P binding domains in glucose phosphate isomerase (GPI) and glutamine-fructose-6-phosphate transaminase 1 (GFPT1) (McFerrin and Atchley 2012).

MondoA and ChREBP are close paralogs sharing extensive homology in their MCR (52% identical), bHLHZip (61% identical), and DCD (70% identical) domains (Billin and Ayer 2006; McFerrin and Atchley 2011). Like MondoA, ChREBP also functions as a glucose sensor and interacts with MLX (Uyeda and Repa 2006; Postic et al. 2007; Havula and Hietakangas 2012). Here we limit our discussion to the functions of the MCRs of MondoA, although the analogous domains in ChREBP seem to function similarly. Initially, MondoA and ChREBP were found to be most highly expressed in skeletal muscle and liver, respectively (Billin et al. 2000; Uyeda and Repa 2006; Postic

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et al. 2007). Given the important role played by these tissues in glucose homeostasis, this expression pattern makes sense from a physiological perspective. However, both MondoA and ChREBP are broadly expressed in most tissues (www.biogps.org) and likely have uncharacterized functions outside of controlling glucose homeostasis.

One unique feature of MondoA:MLX complexes is that they are not constitutively nuclear, like other members of the MYC, MAX, and MXD families. Rather they localize to the outer membrane of the mitochondria (OMM) and shuttle between the OMM and the nucleus (Sans et al. 2006), providing an initial subcellular localization regulatory step. Given the multifaceted role played by mitochondria in bioenergetics, we initially postulated that MondoA:MLX complexes sense some aspect of intracellular energy status and communicate that information to the nucleus to trigger an adaptive transcriptional response. We now know that MondoA:MLX complexes sense glucose and other hexoses and play a predominant role in regulating glucose-induced transcription in murine embryonic fibroblasts (MEFs), HA1ER cells, and a breast epithelial cell line transformed with SV40 large T antigen and activated H-Ras (Stoltzman et al. 2008). ChREBP, which is also held latently in the cytoplasm but not at the OMM, plays a similarly predominant role in controlling glucose-induced transcription in rat hepatocytes (Ma et al. 2006). For both MondoA:MLX and ChREBP:MLX complexes, glucose drives nuclear accumulation and promoter binding of the heterodimers.

How glucose controls the nuclear accumulation and transcriptional activity of MondoA:MLX and ChREBP:MLX complexes is potentially quite complicated and no unifying model has yet emerged. Early models of ChREBP regulation implicated a number of glucose-dependent phosphorylation and dephosphorylation events in controlling ChREBP nuclear activity (Uyeda and Repa 2006; Postic et al. 2007). These early reports are controversial and have been revisited several times (Postic et al. 2007). More recent reports suggest allosteric models in which glucose-derived metabolites

such as glucose 6-phosphate, xylulose 5-phosphate, and fructose 2,6-bisphosphate control the nuclear activity of MondoA and ChREBP (Li et al. 2010; Arden et al. 2012; Dentin et al. 2012; Petrie et al. 2013). Interestingly, target gene selection by MondoA and ChREBP seems to be dictated by specific metabolites. For instance, glucose and the nonglucose hexoses allose and 3-O-methylglucose, induce MondoA binding to the thioredoxin interacting protein (TXNIP) promoter, whereas only glucose induces MondoA binding to the protein targeting to glycogen promoter (Stoltzman et al. 2011; Petrie et al. 2013).

The phosphoacceptor sites identified in ChREBP are not conserved in MondoA, suggesting that MondoA:MLX complexes do not use the same glucose-dependent phosphoregulation. Several lines of evidence support the model that MondoA:MLX complexes sense glucose 6-phosphate (Stoltzman et al. 2008). For example, nuclear accumulation of MondoA:MLX complexes require the enzymatic activity of hexokinases, which phosphorylate glucose to glucose 6-phosphate (G6P) (Stoltzman et al. 2008). Both glucose and 2DG induce the nuclear translocation of MondoA:MLX complexes, but 2DG is more effective than glucose. 2DG is phosphorylated by hexokinases but is not further metabolized by the glycolytic pathway. Thus, phosphorylated 2DG builds up to a significantly higher level than G6P and we proposed that this accounts for the higher activity of 2DG in driving MondoA:MLX to the nucleus. Supporting this hypothesis, cells that lack phosphoglucose isomerase cannot isomerize G6P to fructose 6-phosphate and accumulate G6P. In these cells glucose and 2DG are equally effective at driving nuclear accumulation of MondoA:MLX complexes (Stoltzman et al. 2011). Together these experiments support an important role for G6P. However, metabolites downstream from G6P also regulate MondoA:MLX transcriptional activity. For example, glucosamine 6-phosphate, a metabolite of the hexosamine biosynthetic pathway, can also drive nuclear accumulation of MondoA:MLX complexes (Stoltzman et al. 2011). Determining which metabolites regulate MondoA:MLX complexes directly will almost

certainly require deciphering the mechanistic details in vitro.

Regardless of the exact mechanism(s) by which glucose regulates MondoA:MLX nuclear accumulation, the target is almost certainly the MCRs. For example, deletion of the MCRs renders MondoA:MLX complexes constitutively nuclear and transcriptionally active in the complete absence of glucose. Introducing point mutations in highly conserved residues in individual MCRs fails to phenocopy the deletion of MCR I-V, suggesting that the MCRs function as a structural unit (Fig. 1) (Eilers et al. 2002; Peterson et al. 2010). Further supporting this hypothesis is the fact that the spacing between

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MCRs II–IV is nearly invariant across species. The spacing between MCRI and MCRII and between MCRIV and MCRV is slightly more variable; however, the linear order and presumably the structure of the MCRs are conserved across species (McFerrin and Atchley 2012). Little is known about the function of the individual MCRs. MCRII contains a CRM1-dependent nuclear export signal and MCRIII binds 14-3-3 isoforms. Current data suggest that 14-3-3 binding to MCRIII occludes CRM1 binding to MCRII, resulting in nuclear localization of the MondoA:MLX heterocomplex (Eilers et al. 2002). However, MondoA mutants with mutations in MCRII are constitutively nuclear, yet fail to bind target gene promoters and activate gene expression in the absence of glucose. Thus, nuclear accumulation of MondoA:MLX complexes is not sufficient to activate gene expression (Peterson et al. 2010). Whether MCRI, MCRIV, or MCRV form additional protein partnerships is not known; however, it is possible that they participate in intramolecular interactions that stabilize the structure of the entire MCR domain.

Figure 2. Cellular roles for TXNIP. (A) TXNIP functions in numerous contexts within the cell. Prominent roles include inhibition of the redox protein thioredoxin (TXN), restricting glucose uptake through multiple potential mechanisms, inhibition of mTOR signaling via REDD1 stabilization, and restricting tumor formation. (B) MLXIP-TXNIP signaling serves to restrict cell growth via a metabolic or stress checkpoint. Disruption of this regulatory circuit is one potential mechanism that cells use to transition to an unrestricted growth phenotype, commonly found in cancer cells.

THE MondoA-TXNIP NEGATIVE-FEEDBACK LOOP

The best-characterized glucose-induced direct transcriptional target of MondoA:MLX complexes is TXNIP. TXNIP as its name implies was identified as a binding partner and negative regulator of the small redox protein thioredoxin (TXN) (Chen et al. 2008a; Jin et al. 2011; Zhou et al. 2011; Patwari and Lee 2012). TXNIP has pleiotropic functions in the cell, most prominent among these being the regulation of reactive oxygen species by inhibiting TXN function, and blocking glucose uptake and aerobic glycolysis (Fig. 2A). Recently, TXNIP has been shown to suppress mammalian target of rapamycin (mTOR) activity via stabilization of REDD1 (Jin et al. 2011). TXNIP has also been implicated as a functional component of the NLRP3 inflammasome, required for the cleavage and production of functional IL-1 β (Schroder et al. 2010). TXNIP is thought to be a tumor suppressor in a number of cancers and TXNIP

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knockout mice are prone to develop hepatocellular carcinoma (HCC) spontaneously (Sheth et al. 2006; Zhou et al. 2011). This may reflect TXNIP's proapoptotic activity, its stabilization of the cell-cycle inhibitor p27Kip1 (CDK1B), its destabilization of hypoxia inducible factor 1 α (HIF-1 α), or its negative regulation of glucose utilization (Jeon et al. 2005; Muoio 2007; Parikh et al. 2007; Shin et al. 2008; Stoltzman et al. 2008, 2011). TXNIP also appears to play prominent roles in organismal glucose homeostasis by regulating peripheral glucose uptake and glucose toxicity of pancreatic β cells (Parikh et al. 2007; Chen et al. 2008a). A complete accounting of TXNIP's functions is not possible here, although several recent reviews are available (Kim et al. 2007; Muoio 2007; Zhou et al. 2011; Patwari and Lee 2012; Zhou and Chng 2012).

Multiple experiments illustrate that TXNIP is a direct and glucose-induced target of MondoA. First, TXNIP levels are reduced or not detectable in MondoA knockdown or knockout cells grown in glucose-containing medium. Similarly, glucose induction of TXNIP is reduced or eliminated in MondoA knockdown or knockout cells (Stoltzman et al. 2008, 2011; Kaadige et al. 2009; Chen et al. 2010a; Peterson et al. 2010). Thus, MondoA is required for TXNIP expression under basal glucose conditions and glucose induction. Second, MondoA:MLX complexes accumulate rapidly in the nucleus following glucose addition and this nuclear accumulation is not blocked by the protein synthesis inhibitor cycloheximide. Third, MondoA can be detected on the TXNIP promoter in a region that contains a ChoRE within 15 minutes of glucose addition (Stoltzman et al. 2008). Finally, the activity of a TXNIP luciferase reporter was virtually eliminated by mutating the ChoRE. Collectively, these experiments suggest that the nuclear accumulation of MondoA:MLX and its regulation of TXNIP is an immediate early response to changes in the flux of glucose into the glycolytic pathway.

Among TXNIP's many functions is a blockade of glucose uptake and aerobic glycolysis (Fig. 2A). Thus, the glucose-dependent induction of TXNIP by MondoA:MLX complexes

constitutes a negative-feedback loop that restricts glucose uptake when glycolytic flux is high. A close paralog of TXNIP is arrestin domain-containing protein 4 (ARRDC4), whose expression is also glucose induced in a MondoA:MLX-dependent manner (Kaadige et al. 2009; Peterson et al. 2010). ARRDC4 can also restrict glucose uptake (Patwari et al. 2009), so TXNIP and ARRDC4 likely function in concert or redundantly downstream from MondoA:MLX complexes to restrict glucose availability (Sloan and Ayer 2010). TXNIP may restrict glucose by binding the glucose transporter GLUT1, and internalization of the transporter via clathrin-coated pits (Wu et al. 2013). Several additional potential mechanisms for TXNIP restricting glucose uptake include destabilization of HIF1A, interactions with endosomal sorting complex required for transport (ESCRT) machinery, or control of PTEN activity (Hui et al. 2008; Shin et al. 2008; Rauch and Martin-Serrano 2011). What is clear is that TXNIP can block both insulin-independent and insulin-induced glucose uptake and that this function of TXNIP does not require its interaction with TXN (Patwari et al. 2009). The multiple mechanisms by which TXNIP may restrict glucose uptake suggest that its function may depend on cell context.

Considerable evidence supports a physiological role for the MondoA:MLX-TXNIP negative-feedback loop in restricting glucose uptake. For example, reduction of MLXIP or TXNIP triggers elevated glucose uptake and aerobic glycolysis. Following activation of naïve T cells, TXNIP protein and mRNA expression are restricted after 24 hours and this is concomitant with reprogramming to a highly glycolytic metabolic profile (Elgort et al. 2010). Furthermore, TXNIP induction is required for glucose-induced pancreatic β -cell apoptosis (Chen et al. 2008a). Thus, loss of MondoA or TXNIP is sufficient to trigger metabolic reprogramming toward one of the most common phenotypic features of the transformed state. Further, expression of a dominant active form of MondoA can potentially block glucose uptake and this reduction of glucose uptake is partially dependent on elevated TXNIP levels (Hui et al. 2008;

Kaadige et al. 2009; Stoltzman et al. 2011). Thus, TXNIP is a required effector of MondoA-dependent reduction of glucose uptake, yet other MondoA targets must also be required. ARRDC4 is one obvious candidate. Finally, MondoA and TXNIP are required for the full elaboration of the hexose curb, the restriction of glucose uptake following exposure to different hexose sugars (Stoltzman et al. 2011). The MondoA-TXNIP regulatory circuit is extant in many cell types and is predicted to have growth-suppressive functions. However, MondoA appears to have growth-promoting functions in B-cell acute lymphocytic leukemia (B-ALL), suggesting that its regulation TXNIP may be restricted in some cell types (Wernicke et al. 2012).

GLUTAMINE-DEPENDENT REPRESSION OF TXNIP

In addition to glucose, the amino acid glutamine is also typically required for cell growth (Deberardinis et al. 2008). Much like glucose, glutamine can flux into a number of metabolic or biosynthetic pathways, yet glucose-stimulated induction of TXNIP can be repressed by glutamine. The presence of glutamine effects neither the glucose-induced nuclear accumulation of MondoA:MLX complexes nor their binding to the TXNIP promoter. In contrast, glutamine-dependent repression of the TXNIP promoter can be completely reversed with a histone deacetylase (HDAC) inhibitor, suggesting that glutamine triggers the activation, or the recruitment of a HDAC complex to the TXNIP promoter (Kaadige et al. 2009). Given that MondoA:MLX complexes are such potent activators of TXNIP gene expression, we have proposed that they are required for HDAC targeting, yet it is possible that other transcription factors recruit a HDAC-containing corepressor complex to the TXNIP promoter in the presence of glutamine. In this case, the glutamine-targeted repression would be dominant over the potent MondoA-dependent activation of TXNIP.

As glutamine has several intracellular fates (DeBerardinis and Cheng 2010; Shanware et al. 2011), there are several potential mechanisms

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by which it may drive TXNIP gene repression. Although it is difficult to rule out minor contributions from other pathways, several experiments point to a predominant role for α -ketoglutarate (α -KG). α -KG is generated from glutamine through several distinct pathways. Once glutamine enters the cell it is hydrolyzed to glutamate and an ammonium ion in a reaction catalyzed by glutaminase. Glutamate can be converted to α -KG by one of three mechanisms: (1) glutamate can be converted to α -KG and an ammonium ion in a reaction catalyzed by glutamate dehydrogenase; (2) the amino group of glutamate can be transferred to the keto group of pyruvate to generate α -KG and alanine in a reaction catalyzed by alanine transaminase (GPT); and (3) the amino group of glutamate can be transferred to the keto group of oxaloacetate to generate α -KG and aspartate in a reaction catalyzed by aspartate transaminase (GOT1). Aminooxyacetate (AOA) inhibits GPT and GOT1, blocking the formation of α -KG. AOA effectively inhibits glutamine-dependent repression of TXNIP suggesting a role for α -KG as an important effector in this process. Importantly, cell permeable analogs of α -KG, which restore intracellular α -KG levels in glutamine-depleted cells, completely phenocopy glutamine-dependent repression of TXNIP and bypass the AOA blockade to reduce TXNIP expression. Together, these findings implicate α -KG as a key mediator of glutamine-dependent repression of TXNIP (Kaadige et al. 2009). In each of these experimental treatments, high TXNIP expression tracked with lower glucose uptake. Thus, a final implication of finding that α -KG can repress TXNIP and stimulate glucose uptake is that TCA function may actually lie upstream and modulate glycolysis under some circumstances.

There are several mechanisms by which α -KG may drive repression of TXNIP gene expression. First, as α -KG can replenish the TCA cycle, it is possible that TXNIP promoter activity is directly linked to this central metabolic process (Kaadige et al. 2009). In this regard, the shuttling of MondoA:MLX between the OMM and the nucleus places it in the perfect cellular context to be regulated by mitochondrial cues (Sans

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et al. 2006). Determining the site of action for α -KG-dependent repression of TXNIP and whether α -KG or an α -KG-derived metabolite is sufficient for TXNIP repression will help clarify this mechanism. A second possibility stems from α -KGs role as a cofactor for a number of dioxygenases that have established roles in controlling gene expression or emerging roles in controlling epigenetics (DeBerardinis and Thompson 2012; Yun et al. 2012). This may potentially lead to TXNIP repression through a more indirect mechanism. For example, α -KG is required for the prolyl hydroxylases PHD1 and PHD2, to modify and target HIF1A for degradation. In addition, α -KG is required for the activity of the TET family of DNA demethylases and for the Jumonji family of histone demethylases. Activation of TET demethylases typically results in gene activation, seemingly ruling out this mechanism. In contrast, Jumonji demethylases can contribute to gene activation or repression depending on the histone substrate. Data available from the Santa Cruz genome browser (www.genome.ucsc.edu) shows that the promoter of the TXNIP gene is enriched with histone H3 that is trimethylated at lysine 4 (H3K4me3), an activation mark. This raises the possibility that a histone demethylase from the JARID family, which targets (H3K4me3), could contribute to glutamine-dependent repression of TXNIP. It is also conceivable that an α -KG-dependent dioxygenase modifies MondoA to control its transcriptional activity.

DOES THE MondoA-TXNIP CIRCUIT CONTRIBUTE TO TUMOR SUPPRESSION?

TXNIP has pleiotropic effects in cell physiology and most of these are growth suppressive, suggesting that TXNIP may be a bona fide tumor suppressor. As recently reviewed, TXNIP has been implicated as a tumor suppressor in a number of different cancers including hepatocellular carcinoma, breast cancer, bladder cancer, gastric cancer, and leukemia (Zhou et al. 2011). TXNIP null animals are prone to hepatocellular carcinoma late in life (Sheth et al. 2006), but there are no published studies examining whether TXNIP loss can accelerate tumor-

igenesis driven by other oncogenes. Whether a single function of TXNIP or multiple functions mediate its growth-suppressive function is unknown.

The putative role of TXNIP as a tumor suppressor has been best studied in breast cancer. One study of 788 patients found high TXNIP mRNA expression portended a better prognosis (Cadenas et al. 2010). In a second study, the Chi laboratory identified a protective gene signature that was induced by lactic acidosis (LA), which is a common feature of the tumor microenvironment. TXNIP and ARDC4 were induced by LA exposure and were important components of this protective LA-dependent gene signature (Chen et al. 2008b). In collaboration with the Chi group, we showed that the LA-induced increase in TXNIP was entirely dependent on MondoA and glucose. Further, we used chromatin immunoprecipitation (ChIP) to show that MondoA occupies the TXNIP promoter more robustly under LA growth conditions (Chen et al. 2010a). This finding suggests that LA controls either the amount of MondoA in the nucleus or the DNA-binding activity of MondoA:MLX complexes, rather than controlling the transcriptional activity of MondoA:MLX complexes.

Because TXNIP expression is strictly dependent on MondoA, we have suggested that MondoA may also have a tumor suppressor function in breast cancer. MondoA levels are not generally suppressed in breast cancer, yet given that nuclear and transcriptional activity of MondoA is under complex regulatory control, it is possible that negative regulation of MondoA nuclear activity is more relevant than absolute levels of MondoA protein to a putative growth-suppressive activity. MondoA mRNA levels are suppressed in several types of lymphoma, including Burkitt's lymphoma, which typically has dysregulated MYC expression, superficial bladder cancer, infiltrating bladder urothelial carcinoma, colorectal carcinoma, rectal and colon mucinous adenocarcinoma, prostatic intraepithelial neoplasia, and several types of sarcoma (Fig. 3) (Basso et al. 2005; Detwiller et al. 2005; Sanchez-Carbajo et al. 2006; Tomlins et al. 2007). TXNIP mRNA levels are also generally lower in

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	Cancer	Fold change	P value		Cancer	Fold change	P value
MLXIP down	Superficial bladder cancer (Sanchez-Carbayo et al. 2006)	-4.53	6.65E-12	TXNIP down	Hepatocellular carcinoma (Roessler et al. 2010)	-2.05	1.21E-23
	Infiltrating bladder urothelial carcinoma (Sanchez-Carbayo et al. 2006)	-2.37	3.70E-10		Ovarian serous cystadenocarcinoma (TCGA)	-2.13	3.87E-6
	Prostatic intraepithelial neoplasia (Tomlins et al. 2007)	-2.70	1.53E-6		Chronic lymphocytic leukemia (Haslinger et al. 2004)	-2.28	1.87E-6
	Burkitt's lymphoma (Basso et al. 2005)	-2.50	1.45E-5		Thyroid gland oncocyctic adenoma (Giordano et al. 2006)	-2.50	4.12E-5
	Rectal mucinous carcinoma (TCGA)	-2.22	4.43E-5		Ovarian carcinoma (Bonome et al. 2008)	-3.58	8.80E-7
	Colon mucinous carcinoma (TCGA)	-2.01	1.10E-8		Cutaneous melanoma (Talentov et al. 2005)	-3.01	2.76E-5
					Tongue squamous cell carcinoma (Talbot et al. 2005)	-2.86	8.82E-8
MLXIP up	B-cell acute lymphoblastic leukemia (Haerlach et al. 2010)	3.46	1.32E-58	TXNIP down	Colon adenocarcinoma (TCGA)	-2.85	6.03E-20
	B-cell childhood acute lymphoblastic leukemia (Haerlach et al. 2010)	3.43	1.29E-69		Colon mucinous adenocarcinoma (TCGA)	-3.36	1.08E-13
	Prostate adenocarcinoma (Wallace et al. 2008)	3.63	7.17E-5		Rectal adenocarcinoma (TCGA)	-4.01	1.99E-19
	Clear cell renal cell carcinoma (Jones et al. 2005)	3.08	4.86E-15		Rectal mucinous adenocarcinoma (TCGA)	-3.85	8.03E-5
MLX down	Invasive breast carcinoma (Finak et al. 2008)	-7.05	4.29E-19		Cecum adenocarcinoma (TCGA)	-4.07	1.48E-14
	Colon adenocarcinoma (TCGA)	-2.07	6.02E-15		Squamous cell lung carcinoma (Garber et al. 2001)	-4.88	2.14E-6
	Colon mucinous adenocarcinoma (TCGA)	-2.00	4.16E-13		Lung carcinoid tumor (Bhattacharjee et al. 2001)	-6.62	1.53E-6
	Rectal adenocarcinoma (TCGA)	-2.05	9.06E-15		Dedifferentiated liposarcoma (Barretina et al. 2010)	-2.55	2.94E-12
	Rectal mucinous adenocarcinoma (TCGA)	-2.77	7.43E-5		Pleomorphic liposarcoma (Barretina et al. 2010)	-2.90	3.07E-8
	Cecum adenocarcinoma (TCGA)	-2.34	2.30E-13		Myxoid/round cell liposarcoma (Barretina et al. 2010)	-3.57	3.9E-11
	Acute myeloid leukemia (Stegmaier et al. 2004)	-3.48	4.09E-5		Leiomyosarcoma (Barretina et al. 2010)	-4.26	2.77E-12
					Myxofibrosarcoma (Barretina et al. 2010)	-5.17	2.26E-14
TXNIP up	Benign melanocytic skin nevus (Talentov et al. 2005)	5.94	8.68E-5		Mixed lobular and ductal breast carcinoma (TCGA)	-2.70	8.15E-5
	Diffuse astrocytoma (Sun et al. 2006)	2.61	1.55E-7		Invasive lobular breast carcinoma (TCGA)	-2.87	8.12E-15
	Anaplastic astrocytoma (Sun et al. 2006)	3.94	3.07E-8		Invasive ductal and lobular breast carcinoma (TCGA)	-3.13	9.32E-14
	Oligodendroglioma (Sun et al. 2006)	3.84	1.59E-15		Invasive breast carcinoma (TCGA)	-3.65	6.17E-24
	Brain glioblastoma (Sun et al. 2006)	2.63	1.38E-11		Invasive ductal breast carcinoma (TCGA)	-4.94	5.01E-39
					Mucinous breast carcinoma (TCGA)	-6.05	2.63E-18

Figure 3. MLXIP/MLX/TXNIP expression in tumor data sets. Oncomine (www.oncomine.org) was used to examine changes in MLXIP, MLX, and TXNIP in different cancers. Fold up- or down-regulation is listed along with the *P* value. Following each cancer type is the first investigator of the primary research study. Names listed in parentheses are the individual references. TCGA, The Cancer Genome Atlas.

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many of these same tumors suggesting that the MondoA-TXNIP regulatory circuit may be impaired in these cancer types potentially contributing to their transformed phenotype (Fig. 3) (Bhattacharjee et al. 2001; Garber et al. 2001; Haslinger et al. 2004; Talantov et al. 2005; Talbot et al. 2005; Giordano et al. 2006; Bonome et al. 2008; Barretina et al. 2010; Roessler et al. 2010). However, TXNIP is overexpressed in some cancers suggesting that the role of TXNIP in tumor progression is likely to be context dependent (Talantov et al. 2005; Sun et al. 2006). Perhaps related to this finding, the locus that encompasses the TXNIP gene is amplified in some cancer types (www.cbioportal.org); however, further analysis is required to determine how changes in TXNIP gene copy number relate to TXNIP mRNA and protein expression. Finally, dimerization with MLX is essential for MondoA to access the nucleus and activate gene expression (Peterson et al. 2010). It is intriguing that MLX mRNA levels are reduced in breast and colon cancer and acute myeloid leukemia (Fig. 3) (Stegmaier et al. 2004; Finak et al. 2008), suggesting another route to inactivation of the transcriptional activity of MondoA:MLX complexes and loss of the MondoA-TXNIP circuit.

Most of the current literature supports a growth/tumor suppressive role for the MondoA-TXNIP circuit; however, MondoA is up-regulated in B-ALL, prostate adenocarcinoma, and clear cell renal carcinoma (Fig. 3) (Jones et al. 2005; Wallace et al. 2008; Haferlach et al. 2010). Surprisingly, MondoA promoted glucose uptake in B-ALL cell lines rather than suppressed glucose uptake as shown for the MondoA-TXNIP circuit in other cell types. Further, in a B-ALL cell line, MondoA regulated a “stemness” gene signature and MondoA expression correlated with a more immature and less differentiated cell fate (Wernicke et al. 2012). Interestingly, the gene expression signature generated following MondoA knockdown showed significant overlap with a gene signature that portended a good prognosis for B-ALL patients treated with glucocorticoids (Rhein et al. 2007). This finding contrasts with other reports indicating that TXNIP contributes to glucocorticoid-driven apoptosis in a number of T-cell leu-

kemia cell lines (Wang et al. 2006; Chen et al. 2010b). It may be that in B-ALL, MondoA cannot regulate TXNIP and this tumor/growth-suppressive axis is not intact. These seemingly contradictory findings underscore the necessity of understanding the full spectrum of MondoA targets in different cell lineages in both physiological and pathophysiological states.

METABOLIC REPROGRAMMING IN G₁-INACTIVATION OF THE MondoA-TXNIP CIRCUIT

Once stimulated, quiescent cells must dramatically reprogram their metabolism to support their biosynthetic requirements (Wang and Green 2012) to transit the G₁ phase of the cell cycle and enter S phase. However, cells become independent of both growth factors and nutrients after mid-G₁ (Pardee 1974), indicating that they accumulate sufficient biomolecular precursors to support cell division during G₁. Serum starved, quiescent BJ-TERT cells, an immortal but diploid fibroblast cell line derived from human foreskin, acutely down-regulate the MondoA-TXNIP circuit following serum stimulation, paralleling a rapid up-regulation of glucose uptake and glycolysis, indicative of metabolic reprogramming (Elgort et al. 2010). MondoA-dependent transcriptional activation of TXNIP is blocked by PI3K signaling, whereas translation of preexisting TXNIP mRNA is blocked by Ras signaling. Importantly, constitutive expression of MondoA or TXNIP slows progression into S phase indicating that TXNIP down-regulation is necessary for cells to efficiently transit the G₁ phase and is not just correlated with this transition. This indicates that abrogation of the MondoA-TXNIP circuit is a required component of the metabolic reprogramming toward aerobic glycolysis required to transition from G₀ to S phase, and implies a potential role for MondoA in activation of quiescent stem cell populations.

It is noteworthy that the biosynthetic metabolism of cells in G₁ mirrors that of cancer cells. In normal human fibroblasts, activation of PI3K and Ras contribute to the down-regulation of the MondoA-TXNIP circuit. Given the

preponderance of PI3K and Ras activation in cancer and their established function in driving aerobic glycolysis (Shaw and Cantley 2006), we propose that their suppression of the MondoA-TXNIP circuit may be a general feature of the metabolic phenotype observed in many cancers (Fig. 2B). From a cancer perspective, we suggest that the MondoA-TXNIP regulatory circuit constitutes a metabolic or stress checkpoint that restricts cell growth. In nontransformed cells, MondoA-dependent up-regulation of TXNIP serves to restrict cell growth until the stress can be resolved. In contrast, in cells with constitutive activation of the PI3K pathway and/or Ras, TXNIP levels remain low allowing these cells to bypass this metabolic/stress checkpoint (Fig. 2B).

INTERACTIONS BETWEEN NUTRIENT UTILIZATION AND NUTRIENT AVAILABILITY

MYC has pleiotropic functions in controlling glycolysis, yet its broad reach in controlling cell metabolism extends to mitochondrial activity and glutaminolysis. MYC controls glutaminolysis, in part by up-regulating glutamine transporters and glutaminase, suggesting that MYC overexpressing cells can preferentially use glutamine as a carbon source to fuel biosynthetic reactions (Wise et al. 2008; Gao et al. 2009). Supporting this hypothesis, MYC overexpression renders IMR90 diploid fibroblasts sensitive to glutamine depletion (Yuneva et al. 2007). Further, inhibition of glutaminolysis can inhibit a MYC-dependent B-cell lymphoma model and MYCN-amplified neuroblastomas (Le et al. 2012; Qing et al. 2012). MYCN-amplified neuroblastomas were more sensitive to inhibition of glutaminolysis than nonamplified neuroblastomas, strengthening the connection between MYC activity on glutamine dependence (Qing et al. 2012).

Because MYC overexpression drives glutamine utilization and MondoA represses TXNIP expression in the presence of glutamine, it is possible that the high rate of glutaminolysis in MYC overexpressing cells serves to enhance glutamine-dependent repression of TXNIP. The resulting MYC-dependent reduction in TXNIP

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expression would facilitate aerobic glycolysis to further support cell growth. It is conceivable that the coordinated regulation of TXNIP by MYC and MondoA represents a novel mechanism by which MYC could drive cell growth and macromolecular biosynthesis. At the very least, indirect repression of TXNIP by MYC represents a novel mechanism by which MYC could drive aerobic glycolysis that is independent of its better-documented role in regulating the expression of glycolytic target genes (Kim et al. 2004; Gordan et al. 2007; Dang 2012b). The glutamine-dependent repression of TXNIP we have described is not observed in all cell types (MR Kaadige and DE Ayer, unpubl.), thus it will be of interest to determine whether this regulatory mechanism correlates exclusively with MYC status or whether other regulators also contribute. Triple negative breast cancers have high MYC levels, high rates of aerobic glycolysis, a glycolytic gene signature, and express glutaminase (Chandriani et al. 2009; Foulkes et al. 2010; Palaskas et al. 2011; Horiuchi et al. 2012). Consistent with these findings, triple negative breast cancers are more dependent on glutamine for growth than luminal cancers (Kung et al. 2011). No targeted therapies are currently available for triple negative breast cancers; however, these findings suggest that glutaminase inhibitors, such as BPTES, may have some utility (Robinson et al. 2007).

How restricting glutamine, via glutamine deprivation or inhibition of glutaminolysis, drives apoptosis in a subset of cell types is just becoming clear. In MYCN overexpressing neuroblastoma cells PUMA, NOXA, and TRIB3 are up-regulated following glutamine restriction and work in concert to drive BAX-dependent apoptosis (Qing et al. 2012). Expression of these three effectors is dependent on ATF4, which is selectively translated in a GCN2-dependent manner following glutamine restriction. Although not examined, restricting glutamine would likely increase TXNIP levels in a MondoA-dependent manner. If so, it is possible that TXNIP contributes to apoptosis in glutamine-deprived MYCN-amplified neuroblastoma cells. Whether ATF4 contributes to the activation of TXNIP in the absence of glutamine is

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unknown. However, ER stress, which activates a number of adaptation pathways that stimulate ATF4 translation, also increases TXNIP expression (Lerner et al. 2012; Oslowski et al. 2012). Thus, it will be interesting to determine whether TXNIP, and potentially ARRDC4, contributes to death of glutamine-addicted tumor cells following glutamine restriction.

CONCLUDING REMARKS

MondoA is a highly conserved member of the bHLHZip family of transcription factors, yet we have only a rudimentary understanding of its biological functions. Many of our suppositions regarding MondoA's role in the cell come from its glucose-induced, direct transcriptional regulation of TXNIP and its paralog ARRDC4. In this light, MondoA appears to be a growth suppressor by negatively regulating glucose uptake and aerobic glycolysis. In addition to blocking glucose uptake, TXNIP has additional growth-suppressive functions (e.g., stabilization of CDK1B and destabilization of HIF1A) (Jeon et al. 2005; Shin et al. 2008). We suggest that TXNIP levels may be repressed by MYC-driven glutaminolysis and we have shown that RAS and/or PI3K activation reduce TXNIP levels (Elgort et al. 2010). MYC, RAS, and PI3K are dysregulated in a wide variety of human cancers and each has clear roles in driving increased glucose utilization. Thus, it is possible that down-regulation of TXNIP by these potent oncogenes is widespread and contributes to the metabolic phenotype of the transformed state. However, the recent demonstration that MondoA may play a pro-growth role in B-cell ALL, suggests that its functions are more complicated than this simple model suggests (Wernicke et al. 2012).

Several findings suggest that TXNIP, and likely ARRDC4, are key effectors of MondoA's glucose sensing and adaptation function. Further, the hexose curb requires both MondoA and TXNIP (Stoltzman et al. 2011). However, MondoA knockout MEFs enter quiescence and up-regulate CDK1B in response to serum starvation, whereas TXNIP null MEFs do not (MG Elgort and DE Ayer, unpubl.). Thus, MondoA loss does not phenocopy TXNIP loss, implying

that MondoA has other important transcriptional targets. Our unpublished global expression experiments reveal ~700 MondoA-dependent (regulated more than twofold) transcripts in MEFs grown in the presence of glucose and ~700 MondoA-dependent transcripts in MEFs grown in the absence of glucose. MondoA does enter the nucleus in the absence of glucose (Peterson et al. 2010), so it is not surprising that one can identify MondoA-dependent genes in glucose-free medium. A challenge in the coming years will be to determine the direct MondoA transcriptome under different nutrient conditions.

MYC and MondoA interact genetically in flies showing a synthetic lethal interaction when the gene dose of each is decreased (Billin and Ayer 2006). Whether similar interactions between MYC and MondoA are extant in human cells is not yet known. Given MYC's dysregulation in cancer and the emerging evidence that the MondoA-TXNIP circuit is growth, and potentially, tumor suppressive, understanding whether and how MYC and MondoA interact is an important question. One can envision a model in which MYC and MondoA coordinate nutrient utilization and availability, respectively. If so, unraveling these nutrient utilization and availability pathways may provide novel targets for the treatment of MYC-dependent tumors.

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