Interactions between Myc and MondoA transcription factors in metabolism and tumourigenesis

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Metabolic reprogramming towards aerobic glycolysis is a common feature of transformed cells and can be driven by a network of transcription factors. It is well established that c-Myc and hypoxia-inducible factor-1α (HIF-1α) contribute to metabolic reprogramming by driving the expression of glycolytic target genes. More recently, the c-Myc-related transcription factor MondoA has been shown to restrict glucose uptake and aerobic glycolysis via its induction of thioredoxin-interacting protein (TXNIP). Three recent studies demonstrate that complex and cancer type-specific interactions between c-Myc, MondoA and HIF-1α underlie metabolism, tumourigenesis and drug response. In triple-negative breast cancer, c-Myc blocks MondoA-dependent activation of TXNIP to stimulate aerobic glycolysis. In contrast, in neuroblastoma, N-Myc requires MondoA for metabolic reprogramming and tumourigenesis. Finally, the therapeutic response of BRAFV600E melanoma cells to vemurafenib requires downregulation of c-Myc and HIF-1α and upregulation of MondoA-TXNIP, and the subsequent reprogramming away from aerobic glycolysis. In this minireview we highlight the findings in these three studies and present a working model to explain why c-Myc and MondoA function cooperatively in some cancers and antagonistically in others.

THE PLAYERS AND THEIR ROLES

The MYC family of proto-oncogenes (c-Myc, L-Myc, N-Myc or collectively Myc) encode basic helix-loop-helix leucine zipper (bHLHZip) transcription factors that have roles in normal and oncogenic physiology (Eilers and Eisenman, 2008; Meyer and Penn, 2008). Aberrant Myc activity leads to increased cell proliferation and growth and Myc family members are commonly dysregulated in cancer. Cancers of many different tissue types have dysregulated Myc and, when paired with other mutations, Myc can drive tumourigenesis. Myc’s normal and pathological functions have been the subject of many excellent reviews and will not be discussed here. Rather, we focus on the interplay between Myc and related bHLHZip factors that comprise an extended Myc network. This extended Myc network functions to match nutrient availability with nutrient utilisation, supporting cell growth and tumourigenesis.

Myc requires dimerisation with another bHLHZip transcription factor, Max, for DNA binding and the recruitment of transcriptional machinery. All Myc paralogues dimerise with Max and the different heterocomplexes have many common target genes, although each heterocomplex has a unique subset of target genes and expression patterns. Generally, Myc/Max complexes influence cell growth by driving the expression genes involved in key biosynthetic pathways and by supporting glycolysis and glutaminolysis (Dang, 2012b). Myc can also act as a transcriptional repressor at certain promoters that is critical to its function in driving cell growth and proliferation (Walz et al, 2014).

A complete functional characterisation of Myc proteins is difficult because they do not function in isolation (O’Shea and Ayer, 2013). Rather, their activity is potentially regulated by a dizzying array of additional protein partnerships mediated by families of related bHLHZip transcription factors. The first level of regulation is mediated by the Mad family of transcriptional repressors (Mxd1, Mxi1, Mxd3, Mxd4) that also interact with Max.
Furthermore, Max can also interact with Mnt, which is related to the Mad family, and Mga, which has a T-Box DNA-binding domain in addition to its bHLHZip domain (Hurlin and Huang, 2006). The Mxd/Max complexes repress many of the same targets that are activated by Myc/Max complexes (Iritani et al, 2002); however, no comprehensive genome-wide occupancy studies have been conducted. The second level of regulation is mediated by a Max-like bHLHZip protein called Mlx. Mlx can interact with Mxd1 and Mxd4 and potentially Mnt. Mxd/Mlx, Mxd/Max and Myc/Max all bind similar DNA sites in vitro, suggesting overlapping transcriptional targets in vitro, but again no comprehensive genome-wide study is available (O'Shea and Ayer, 2013). The third level of regulation is that Mlx interacts with the Mondo family of bHLHZip transcriptional activators. There are two Mondo paralogues, MondoA (or MlxIP) and carbohydrate response element-binding protein (ChREBP, MondoB or MlxIPL) (Figure 1). Mondo/Mlx complexes are important nutrient sensors and share a similar DNA-binding sequence with the rest of the network heterocomplexes (O'Shea and Ayer, 2013). The existence of multiple Myc-related bHLHZip factors, their assembly into different heterocomplexes and the participation of these heterocomplexes in potentially overlapping networks suggests that functional output of Myc/Max complexes is likely dictated by many mechanisms: (1) competitive or collaborative interactions with other network complexes at shared targets, (2) competition for limiting Max or Mlx or (3) different complexes may regulate distinct effector pathways that compete or collaborate to control cell growth and tumorigenesis. In this review we focus on the last possibility as three recent papers demonstrate that Myc/Max and MondoA/Mlx complexes play critical roles in matching the cellular demand for nutrients with nutrient availability (Parmenter et al, 2014; Carroll et al, 2015; Shen et al, 2015).

THE MONDO FAMILY

In normal physiology, MondoA and ChREBP are ubiquitously expressed with the highest expression in skeletal muscle and liver, respectively, and are important regulators of glucose-dependent transcription (Peterson and Ayer, 2011). MondoA/Mlx localise primarily to the cytoplasm and translocate to the nucleus in response to increased extracellular glucose concentrations. The mechanistic details of how the heterocomplex senses extracellular glucose are still being worked out, but current data suggest that MondoA/Mlx complexes sense intermediates in the upper portion of the glycolytic pathway (O'Shea and Ayer, 2013). For MondoA, an allosteric mechanism where a glucose-derived metabolite binds directly to the conserved N-terminus to trigger nuclear accumulation, promoter binding and transcriptional activation of target genes seems to be key (Peterson et al, 2010). The transcriptional activity of MondoA/Mlx complexes also requires mitochondrial electron transport (Yu et al, 2010) that enables their nuclear import and promoter binding. Thus, MondoA/Mlx complexes sense the status of two key bioenergetic pathways, allowing cells to adapt to changes in glycolytic flux or mitochondrial activity.

The most well-characterised direct and glucose-dependent target gene of MondoA/Mlx is thioredoxin-interacting protein (TXNIP), a member of the z-arrestin family (O'Shea and Ayer, 2013). TXNIP has pleiotropic functions in different cellular stress response pathways, with one of its most striking features being a potent negative regulator of glucose uptake. It can suppress both insulin-dependent and insulin-independent glucose uptake by limiting the localisation of glucose transporters to the plasma membrane (Wu et al, 2013). Furthermore, in some cell types TXNIP can drive degradation of hypoxia-inducible factor-1z (HIF-1z) (Shin et al, 2008), a central regulator of glycolytic gene expression under hypoxic growth conditions. Thus, the glucose-stimulated and MondoA-dependent induction of TXNIP triggers a negative feedback loop that restricts glucose uptake until glucose homeostasis can be restored. Furthermore, the dependence of the MondoA/TXNIP axis on electron transport ensures that cells with defective oxidative phosphorylation maintain a high glycolytic rate to support cellular bioenergetics.

Given that TXNIP is a potent negative regulator of glucose uptake and aerobic glycolysis, it is not surprising that its expression is reduced in many cancer types and low TXNIP expression correlates with poor clinical outcome in several cancers including breast and gastric cancer (Shin et al, 2008; Chen et al, 2010; Shen et al, 2015). MondoA is absolutely required for TXNIP expression, yet MondoA is not a frequent target for mutational inactivation in cancer. Rather, the activity of MondoA at the TXNIP promoter is suppressed by activation of common oncogenic or pro-growth pathways, for example Ras, PI3K and mTOR (Elgort et al, 2010; Kaadige et al, 2015). The prevalence of Ras, PI3K and mTOR activation across cancer types suggests broad suppression of the MondoA/TXNIP axis in tumourigenesis.

THE EXPANDED NETWORK IN TRIPLE-NEGATIVE BREAST CANCER

The triple-negative breast cancer (TNBC) accounts for 15–20% of breast cancer cases. Typically, TNBCs occur earlier in life, reoccur more frequently and have decreased overall survival. The TNBCs lack expression of the oestrogen receptor (ER), progesterone receptor (PR) and HER2, and hence the 'triple-negative' nomenclature (Foulkes et al, 2010). The TNBCs are more metabolic than other breast cancer subclasses, and thus understanding how nutrients support their growth and survival may provide pathways for therapeutic development. Consistent with Myc's role in regulating aerobic glycolysis, c-Myc expression correlates with the glycolytic phenotype of TNBCs (Palaskas et al, 2011). In contrast, there is no general correlation between c-Myc levels or Myc activity and glutamine utilisation or glutamine-dependence in TNBC (Timmerman et al, 2013; Shen et al, 2015). These findings prompted us to study how c-Myc controls glucose metabolism in TNBC (Shen et al, 2015). As expected, c-Myc knockdown reduced...
glucose uptake in multiple TNBC cell lines. Surprisingly, TXNIP was induced in c-Myc knockdown TNBC cells and its induction was required for the reduction in glucose uptake resulting from c-Myc knockdown. This finding suggests that in addition to its canonical function of driving the expression of glycolytic target genes, c-Myc can also drive glucose metabolism by repressing TXNIP. Mechanistically, c-Myc can bind to proximal double E-box carbohydrate response element (ChoRE) in the TXNIP promoter, also the known MondoA/Mlx binding site. Furthermore, TXNIP induction following c-Myc knockdown is entirely dependent on MondoA. Thus, either c-Myc recruits dominant transcriptional repression machinery to the TXNIP promoter or displaces strongly activating MondoA/Mlx complexes from the TXNIP promoter by direct competition. MondoA levels increase at the TXNIP promoter following c-Myc knockdown, supporting the second model.

In the TNBC line MDA-MB-157, Myc-driven expression of glycolytic target genes and its repression of TXNIP contribute to Myc-driven glucose metabolism to approximately the same extent (Shen et al., 2015). Further studies are needed to determine whether these mechanisms are similarly balanced in other cancer types or whether cancer type-specific mechanisms dictate their relative contributions. Elevated c-Myc drives expression of genes that encode many biosynthetic pathways placing a demand on biosynthetic precursors such as glucose and glutamine (Dang, 2012a). In TNBC, c-Myc-dependent repression of TXNIP increases glucose uptake that helps to match nutrient demand with nutrient availability. A c-Mychigh/TXNIPlow gene signature correlates with reduced overall survival and reduced metastasis-free survival in breast cancer, particularly in TNBC, highlighting the clinical significance of c-Myc-dependent repression of TXNIP (Shen et al., 2015).

It is striking that the c-Mychigh/TXNIPlow signature only correlates with poor clinical outcome in TNBC and not in other subtypes of breast cancer. Furthermore, the gene signature only correlates with poor clinical outcome in tumours that also harbour mutations in the tumour suppressor gene TP53 (Shen et al., 2015). TP53 is mutated in between 60% and 90% of TNBCs (Turner et al., 2013), and this may explain why c-Mychigh/TXNIPlow only correlates with poor clinical outcome in this breast cancer subtype. Thus, there may be functional cooperation between c-Mychigh/TXNIPlow and mutation of TP53. Importantly, this cooperation does not appear to depend on established connections between dysregulated c-Myc expression and TP53 mutation (Shen et al., 2015). Thus, in TNBC, c-Myc and MondoA, via their opposing regulation of TXNIP expression, function antagonistically.

THE EXPANDED NETWORK IN BRAFV600 MELANOMA

c-Myc and MondoA antagonism also appears to be at play in melanomas that harbour the activated alleles of the BRAF kinase (Parmenter et al., 2014). The BRAF-mutant melanomas are highly glycolytic and their sensitivity to the BRAF inhibitor vemurafenib correlates with a dramatic suppression of glucose uptake and aerobic glycolysis. Vemurafenib treatment reduces the membrane expression of the GLUT1 and GLUT3 glucose transporters and decreases expression of hexokinase II, the first rate-limiting enzyme in glycolysis. Importantly, BRAFV600 melanomas rendered resistant to vemurafenib by expression of NRASQ61K do not reprogramme their metabolism in response to drug treatment. Vemurafenib treatment of BRAFV600 melanomas drives upregulation of TXNIP and its parologue ARRD4C that contributes to its suppression of glucose uptake and aerobic glycolysis (Parmenter et al., 2014). MondoA occupancy at the TXNIP and ARRD4C promoters increases in response to vemurafenib treatment and this increase is blocked by expression of NRASQ61K. As expected, MondoA is required for upregulation of TXNIP in response to vemurafenib. MondoA is also required for the decrease in glucose uptake and cell proliferation driven by vemurafenib. These results strongly implicate the MondoA/TXNIP axis as a key effector of the vemurafenib therapeutic response.

Vemurafenib treatment also results in dramatic downregulation of c-Myc and HIF-1z. Furthermore, functional experiments show that downregulation of c-Myc and HIF-1z is required for vemurafenib to suppress glucose metabolism in BRAFV600 melanomas. Unlike TNBCs, where c-Myc knockdown is sufficient to upregulate TXNIP, c-Myc knockdown in one BRAFV600 melanoma cell line only increases TXNIP slightly (Parmenter et al., 2014). Thus, other mechanisms, in addition to c-Myc downregulation, must also contribute to vemurafenib-dependent induction of TXNIP. Together, these experiments establish that activation of the MondoA/TXNIP axis along with reduction of c-Myc and HIF-1z are required in combination for vemurafenib to reprogramme metabolism away from glucose metabolism and suppress growth in BRAFV600 melanomas. Given these findings, it is not surprising that vemurafenib-treated melanomas become dependent on glutamine (Hernandez-Davies et al., 2015). Together, these studies suggest that c-Myc and MondoA work in opposition in these cancers.
apparent functional differences in the extended Myc network in TNBC and neuroblastoma, this same gene signature does not correlate with clinical outcome in invasive breast carcinoma.

CONCLUSIONS

These three studies highlight the complex interactions between the members of the extended Myc network in metabolism and tumourigenesis (Parmenter et al., 2014; Carroll et al., 2015; Shen et al., 2015). The studies in TNBC and BRAFV600 melanoma suggest that MondoA and Myc function antagonistically in controlling glucose uptake/glycolysis. In contrast, in neuroblastoma, N-Myc amplification drives a dependence on MondoA and Mlx (Figure 2). These findings seem contradictory, but we suggest that the activities of Myc and MondoA function principally to match the demand for biosynthetic precursors with their availability. We speculate that in TNBC, TXNIP suppression by Myc increases the availability of glucose to support Myc-driven biosynthetic reactions. In contrast, in neuroblastoma, N-Myc increases MondoA levels that in turn activate glutaminolysis and lipogenesis to support N-Myc-driven biosynthetic reactions. Together, these data suggest that in cells or tumours that depend primarily on glucose catabolism to fuel biosynthesis, Myc and MondoA will function antagonistically. In contrast, in cells or tumours that depend primarily on catabolism of glutamine and lipids to fuel biosynthesis, Myc and MondoA will function collaboratively.

How broadly the complex interplay between Myc and MondoA extends across tumourigenesis remains to be explored. Current data suggest that the primary function of the MondoA/TXNIP axis is in growth and tumour suppression. For example, TXNIP is downregulated in many tumour types and is only infrequently upregulated in tumours (O’Shea and Ayer, 2013). Furthermore, MondoA is not strongly downregulated in tumours, but several common oncogenic drivers suppress its transcriptional activity at the TXNIP promoter. It will be interesting to determine whether tumours that show suppression of the MondoA/TXNIP axis are particularly dependent on glucose. MondoA has been proposed to function as an oncogene in B-cell acute lymphoblastic leukaemia (Wernicke et al., 2012). The function of c-Myc in B-ALL is not well studied, but elevated c-Myc levels do associate with increased risk of persistent disease (Allen et al., 2014). Whether MondoA is required for B-ALL growth and tumourigenesis or is required to support other c-Myc-driven B-cell lymphomas such as Burkitt’s and diffuse large B-cell should be investigated (Slack and Gascoyne, 2011). As argued above, these Myc-driven tumours may be particularly dependent on glutamine or lipid catabolic pathways.

The highlighted studies indicate that Myc/Max complexes have cell-type differences in their transcriptional targets. For example, in neuroblastoma, N-Myc/Max increases the expression of MondoA and TXNIP (Carroll et al., 2015), whereas in TNBC/ c-Myc represses TXNIP without influencing MondoA levels (Shen et al., 2015). These cell-type differences may reflect differences in the plett of co-activators/co-repressors available in a given cell type, or they may reflect more indirect mechanisms such as c-Myc- or MondoA-driven changes in cell metabolism. Furthermore, the relationship between MondoA and Myc may be influenced by the relative level of Myc amplification and/or the specific Myc homologue that is deregulated (Murphy et al., 2008). With the discovery of Max almost 25 years ago and the demonstration that Myc/Max complexes functioned as transcriptional activators, there was hope that determining ‘how’ c-Myc functioned in normal and pathological settings would follow directly from determining the cohort of Myc/Max transcriptional targets. However, many studies now highlight, studying Myc in isolation provides only partial answer to what is a complex multidimensional puzzle.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Allen A, Gill K, Hoehn D, Sulis M, Bhagat G, Alobeid B (2014) C-myc protein expression in B-cell acute lymphoblastic leukemia, prognostic significance? Leuk Res 38: 1061–1066.
Carroll PA, Diolaiti D, Mcferrin L, Gu H, Djukovic D, Du J, Cheng PF, Anderson S, Ulrich M, Hurley JB, Raftery D, Ayer DE, Eisenman RN (2015) Deregulated Myc requires MondoA/Mlx for metabolic reprogramming and tumorigenesis. Cancer Cell 27: 271–285.
Chen JL, Merl D, Peterson CW, Wu J, Liu PY, Yin H, Muoio DM, Ayer DE, West M, Chi JT (2010) Lactic acidosis triggers starvation response with paradoxical induction of TXNIP through MondoA. PLoS Genet 6: e1001093.
Dang CV (2012a) Links between metabolism and cancer. Genes Dev 26: 877–890.
Dang CV (2012b) MYC on the path to cancer. Cell 149: 22–35.
Eilers M, Eisenman RN (2008) Myc’s broad reach. Genes Dev 22: 2755–2766.
Elgort MG, O’Shea JM, Jiang Y, Ayer DE (2010) Transcriptional and translational downregulation of thioredoxin interacting protein is required for metabolic reprogramming during G1. Genes Cancer 1: 893–907.
Foulkes WD, Smith IE, Reis-Filho JS (2010) Triple-negative breast cancer. N Engl J Med 363: 1938–1948.
Hernandez-Davies JE, Tran TQ, Reid MA, Rosales KR, Lowman XH, Pan M, Moriceau G, Yang Y, Wu J, Lo RS, Kong M (2015) Vemurafenib resistance reprograms melanoma cells towards glutamine dependence. J Transl Med 13: 310.
Hurlin PJ, Huang J (2006) The MAX-interacting transcription factor network. Semin Cancer Biol 16: 265–274.
Iritani BM, Dehrow I, Grandori C, Gomez I, Klacking M, Carlos IS, Eisenman RN (2002) Modulation of T-lymphocyte development, growth and cell size by the Myc antagonist and transcriptional repressor Madl. EMBO J 21: 4820–4830.
Kaadige MR, Yang J, Wilde BR, Ayer DE (2015) MondoA-Mlx transcriptional activity is limited by mTOR-MondoA interaction. Mol Cell Biol 35: 101–110.

Louis CU, Shohet JM (2015) Neuroblastoma: molecular pathogenesis and therapy. Annu Rev Med 66: 49–63.

Meyer N, Penn LZ (2008) Refocusing on 25 years with MYC. Nat Rev Cancer 8: 976–990.

Murphy DJ, Junttila MR, Pouyet L, Karnezis A, Shchors K, Bui DA, Brown-Swigart L, Johnson L, Evan GI (2008) Distinct thresholds govern Myc’s biological output in vivo. Cancer Cell 14: 447–457.

O’Shea JM, Ayer DE (2013) Coordination of nutrient availability and utilization by MAX- and MLX-centered transcription networks. Cold Spring Harb Perspect Med 3: a014258.

Palaskas N, Larson SM, Schultz N, Komisopoulou E, Wong J, Rohle D, Campos C, Yannuzzi N, Osborne JR, Linkov I, Kastenhuber ER, Tascchereau R, Plaisier SB, Tran C, Heguy A, Wu H, Sander C, Phelps ME, Brennan C, Port E, Huse JT, Graeber TG, Mellinghoff IK (2011) 18F-Fluorodeoxy-glucose positron emission tomography marks MYC-overexpressing human basal-like breast cancers. Cancer Res 71: 5164–5174.

Parmenter TJ, Kleinschmidt M, Kinross KM, Bond ST, Li J, Kaadige MR, Rao A, Sheppard KE, Hugo W, Pupo GM, Pearson RB, Mcgce SL, Long GV, Scodyer RA, Rizos H, Lo RS, Cullinane C, Ayer DE, Ribas A, Johnstone RW, Hicks RJ, Mcarthur GA (2014) Response of BRAF-mutant melanoma to BRAF inhibition is mediated by a network of transcriptional regulators of glycolysis. Cancer Discov 4: 423–433.

Peterson CW, Ayer DE (2011) An extended Myc network contributes to glucose homeostasis in cancer and diabetes. Front Biosci (Landmark Ed) 16: 2206–2223.

Peterson CW, Stoltzman CA, Sighinolfi MP, Han KS, Ayer DE (2010) Glucose controls nuclear accumulation, promoter binding, and transcriptional activity of the MondoA-Mlx heterodimer. Mol Cell Biol 30: 2887–2895.

Shen L, O’Shea JM, Kaadige MR, Cunha S, Wilde BR, Cohen AL, Welm AL, Ayer DE (2015) Metabolic reprogramming in triple-negative breast cancer through Myc suppression of TXNIP. Proc Natl Acad Sci USA 112: 5425–5430.

Shin D, Jeon HJ, Jeong M, Suh HW, Kim S, Kim HC, Moon OS, Kim YS, Chung JW, Yoon SR, Kim WH, Choi I (2008) VDUP1 mediates nuclear export of HIF1alpha via CRM1-dependent pathway. Biochim Biophys Acta 1783: 838–848.

Slack GW, Gascoyne RD (2011) MYC and aggressive B-cell lymphomas. Adv Anat Pathol 18: 219–228.

Timmerman LA, Holton T, Yuneva M, Louie RJ, Padro M, Daemen A, Hu M, Chan DA, Ethier SP, Van’T Veer LJ, Polyak K, Mccormick F, Gray JW (2013) Glutamine sensitivity analysis identifies the xCT antiporter as a common triple-negative breast tumor therapeutic target. Cancer Cell 24: 450–465.

Turner N, Moretti E, Siclari O, Migliaccio I, Santarpia L, D’inalci M, Piccolo S, Veronesi A, Zambelli A, Del Sal G, Di Leo A (2013) Targeting triple negative breast cancer: is p53 the answer? Cancer Treat Rev 39: 541–550.

Waltz S, Lorenzin F, Morton J, Wiese KE, Von Eyss B, Herold S, Ryck L, Dumay-Odelot H, Karim S, Bartkuhn M, Roels F, Wustefeld T, Fischer M, Teichmann M, Zender L, Wei CL, Sansom O, Wolf E, Eilers M (2014) Activation and repression by oncogenic MYC shape tumour-specific gene expression profiles. Nature 511: 483–487.

Wernicke CM, Richter GH, Beinvogl BC, Pfehm S, Schlitter AM, Bandapalli OR, Prazeres Da Costa O, Hattenhorst UE, Volkmer I, Staeger MS, Esposito I, Burdach S, Grunewald TG (2012) MondoA is highly overexpressed in acute lymphoblastic leukemia cells and modulates their metabolism, differentiation and survival. Leuk Res 36: 1185–1192.

Wu N, Zheng B, Shaywitz A, Dagon Y, Tower C, Bellinger G, Shen CH, Wen J, Asara J, Mgraw TE, Kahn BB, Cantley LC (2013) AMPK-dependent degradation of TXNIP upon energy stress leads to enhanced glucose uptake via GLUT1. Mol Cell 49: 1167–1175.

Yu FX, Chai TF, He H, Hagen T, Luo Y (2010) Thioredoxin-interacting protein (Txnip) gene expression: sensing oxidative phosphorylation status and glycolytic rate. J Biol Chem 285: 25822–25830.