Title: MAX and MYC: a heritable break up

Alberto Cascón[1,2] and Mercedes Robledo[1,2]

[1]Hereditary Endocrine Cancer Group, Spanish National Cancer Research Centre (CNIO), Madrid, Spain

[2]ISCIII Center for Biomedical Research on Rare Diseases (CIBERER)

Address correspondence to: Alberto Cascón or Mercedes Robledo

Hereditary Endocrine Cancer Group, Human Cancer Genetics Programme

Centro Nacional de Investigaciones Oncológicas, Melchor Fernández Almagro 3, 28029 Madrid, Spain

Phone: +34-91-224 69 47

Fax: +34-91-224 69 23

e-mail: acascon@cnio.es; mrobledo@cnio.es

ABSTRACT

The overexpression of MYC, which occurs in many tumors, dramatically disrupts the equilibrium between activation and repression of the oncogenic MYC/MAX/MXD1 network, favoring MYC-MAX complexes and thereby impairing differentiation and promoting cell growth. While for some time it has appeared that MAX was necessary for both the activation and repression of the axis, recent evidence has shown that MYC retains considerable biological function in the absence of MAX. The presence of germline *MAX* mutations in patients with hereditary pheochromocytoma is testament to the predominant role of MAX as a negative regulator of the network, and suggests that MYC deregulation plays a role in hereditary cancer predisposition. This finding also confirms the importance of impairment of the MYC/MAX/MXD1 axis in the development of aggressive neural tumors, since MYCN overexpression is an established genetic hallmark of malign neuroblastoma, and it is likely that MXI1 plays a relevant role in the development of medulloblastoma and glioblastoma. Finally, the likely malignant behavior of tumors with mutations in *MAX* points to MYC as a candidate therapeutic target in the treatment of metastatic pheocromocytoma.

Introduction

MAX (MYC associated protein X) is a ubiquitous, constitutively expressed protein that plays a central role in the control of the MYC/MAX/MXD1 axis, one of the better known cellular networks whose deregulation contributes to the genesis of many human cancers. MAX was the first-described *bona fide* MYC-interacting protein, and it appears that it is an essential dimerization partner for the other members of the axis to be active. Thus, while MYC activates transcription binding to E-box DNA recognition sequences in target gene promoters through heterodimerization with MAX, heterodimers of MAX with MXD1 family members (MXD1, MXI1, MXD3 and MXD4), MNT and MGA antagonize MYC-dependent cell transformation by transcriptional repression of the same E-box target DNA sequences (1). In addition, MAX is the only member of the network that homodimerizes efficiently, although the biological role of these dimers remains unknown. The promiscuous interactions of MAX with MYC and MYC repressors maintain a complex equilibrium that dictates whether the transcription of thousands of target genes is activated or repressed. This dual task of MAX has probably led to the assumption that MAX integrity is necessary not only for correct cell behavior but also for tumor homeostasis, and therefore that it is unlikely that mutations affecting *MAX* could occur in cancer.

MAX and cancer

Recently, MAX has gained special prominence in human genetics since germline loss-of-function mutations in the *MAX* gene have been reported in patients with hereditary pheochromocytoma (PCC) (2), a rare neural crest cell tumor localized mainly within the adrenal medulla which rarely metastasizes. Prior to this, 30–40% of patients with PCC were thought to be hereditary (3), with autosomal inheritance caused by germline mutations affecting one of nine susceptibility genes: *RET*, *VHL*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *NF1*, and *TMEM127*. Mutations in *MAX* were identified by sequencing the entire exome of three unrelated patients with a family history of PCC who did not have mutations in

any of the known susceptibility genes. The patients were selected for the study because their tumors had very homogeneous expression profiles in a previous transcriptional study (4). The expression profile differentiated the three MAX tumors from all other PCCs, with or without germline alterations in the other PCC susceptibility genes, and suggested that they might have the same genetic cause. The presence of germline mutations in the patients, as well as the loss of heterozygosity of the wild-type allele and the absence of protein in the tumors, demonstrated that *MAX* is a tumor suppressor gene that causes hereditary PCC. Further analysis of 59 patients, chosen because they had bilateral PCC and/or age of onset less than thirty years, led to the detection of 5 additional cases with mutations in *MAX*. Most of the mutations affected highly conserved amino acids within the bHLHZip (basic helix-loop-helix leucine zipper) domain of MAX. The bHLHZip domain is the common structural element among the members of this transcriptional network and is responsible for the specificity and stability of homo- and heterodimer formation (HLHZip), and for DNA recognition via interactions of the basic region (b) and the major groove (5). The presence of alterations affecting this critical domain involved in protein:protein interactions and DNA binding, presumably destroy the ability of MAX to antagonize MYC-dependent cell transformation, leading to tumor development.

It has long been known that a homozygous chromosomal alteration involving the *MAX* gene leads to a protein incapable of homo- or heterodimerization, and therefore incapable of repressing transcription from E-box elements in PC12 cells (derived from rat adrenal PCC); it has been shown that reintroduction of MAX in PC12 cells results in transcriptional repression and reduction in growth rate (6). This finding both confirmed the role of MAX in the regulation of MYC-dependent transcriptional activation and revealed that MYC may also function, at least in PC12 cells, in the absence of normally functioning MAX protein (6). The ability of MYC to function independently of MAX was later demonstrated in *Drosophila* (reviewed in (7)), suggesting that MAX´s pivotal role in the MYC/MAX/MXD1 network is more related to repression than to activation. The involvement of *MAX* in the genetic susceptibility to PCC in

humans also suggests that the loss of MAX in PC12 cells contributes to the histogenesis of the primary tumors rather than to the establishment of the cell line.

The presence of *MAX* mutations in patients with hereditary PCC highlights the importance of the MYC/MAX/MXD1 network in the development of neural crest tumors. Amplification and overexpression of MYCN are well-known genetic hallmarks in neuroblastoma (8), a neural crest cell pediatric tumor originating primarily in the adrenal gland that is the second most incident of all solid tumors in children. MYCN amplification, leading to mRNA and protein overexpression, occurs in 20-25% of neuroblastomas and is strongly associated with advanced disease stages and rapid tumor progression (9). The involvement of MYCN expression in the biological behavior of these tumors remains unclear, but it has been recently reported that high-level expression of MYCN in neuroblastomas lacking amplification of the MYCN locus results in a benign phenotype (10). Thus, MYCN overexpression leads to a more tumorigenic phenotype, but the degree of aggressiveness appear to depend directly on MYCN copy number. Ablation of MAX´s transcriptional repression of MYC in PCC could lead to the same oncogenic MYC deregulation that occurs in neuroblastoma. In this regard, the observation that 25% of patients with mutations in *MAX* develop metastasis suggests that a correlation exists between MAX loss-of-function and metastatic potential. The malignant behavior of MAX-related tumors is congruous with what is known about neuroblastomas and adds weight to the idea that alterations in the main regulator of MYC could lead to the same effect in PCC that the amplification of MYCN provokes in neuroblastoma (i.e. highly malignant disease and poor prognosis). It is also noteworthy that the second hit observed for MAX-related PCCs was the loss of heterozygosity, either by uniparental disomy (UPD) or loss of chromosome 14q, where MAX is located (2). This is especially remarkable since loss of chromosome 14q23-q32 has been described in 22% of primary neuroblastomas, and was inversely correlated with MYCN amplification (11). In addition, 14q-UPD has been recently reported in neuroblastomas (12), and a significant downregulation of two microRNAs (miR-487b and miR-410) located on

14q32 have been also associated with high-risk neuroblastomas (13). These findings suggest that *MAX* could be the target of this chromosomal loss and that the deregulation of MYCN observed in neuroblastoma occurs either through direct overexpression of the protein or due to lack of MAX-related repression. It is noteworthy that no neuroblastomas were found in *MAX* mutation carriers (2), although the small number of patients studied meant that no definitive conclusion could be reached regarding MAX´s participation in neuroblastoma development. In addition, given that ubiquitous nature of MAX expression and MAX´s key role as regulator of this axis, one might expect that patients with germline mutations in this gene develop other tumors as well. The analysis of a larger series of *MAX* mutation carriers will provide further information about the overall involvement of *MAX* alterations in cancer predisposition.

MYC and cancer

Many, if not most, human tumors present with elevated levels of MYC. Indeed, important aspects of tumor biology such as proliferation, cell adhesion and angiogenesis are affected by enhanced expression of MYC, which makes deregulation of this oncogene a hallmark of cancer. Under normal conditions, the stimulation of cells by internal and external signals leads to a rapid and strong over-expression of MYC, MYCN and MYCL1 mRNA and protein that persists into the cell cycle, and subsequently declines to low levels in quiescent cells (14). In addition, MYC overexpression sensitizes primary cells to apoptosis in response to growth factor withdrawal, which might provide protection against the potentially detrimental activity of MYC in terms of tumorigenesis (15). The oncogenic activity of MYC proteins begins when their normal transcriptional regulation is disrupted by gene amplification and translocation, which then leads to abnormally increased levels of intracellular MYC. Overexpression of MYC promotes oncogenic transformation and tumorigenesis by on the one hand activating the transcription of target genes that drive cell proliferation and stimulate angiogenesis, and on the other repressing cell differentiation (16). It is noteworthy that while

mutations within the open reading frame of MYC are infrequent, it is widely accepted that MYC deregulation is not solely restricted to translocations and amplifications at the MYC locus, and this suggests that the impact of MYC deregulation on human cancer incidence is greater than previously thought. Regarding cancer predisposition, inactivating germline mutations in *MYCN* have been found in patients with Feingold syndrome and, as expected, carriers of heterozygous *MYCN* alterations did not develop tumors (17). However, the recently reported contribution of *MAX* germline mutations to cancer susceptibility highlights the importance of MYC regulation in hereditary malignancies, and opens up the possibility of finding new alterations in other bHLHZip repressors of the network that are involved in tumorigenesis through their failure to repress MYC.

MYC/MAX interacting proteins and cancer

The members of the MXD1 (MAX dimerization protein 1) family, MXD1, MXI1, MXD3 and MXD4, have been shown in vivo to promote differentiation, block cellular growth and MYC-induced transformation, and suppress the development of cancer (18). In addition, MNT (MAX binding protein) is likely to be a key regulator of MYC activity, and MGA (MAX gene associated), whose biological function remains unknown, appears to contribute to the silencing of E2F- and MYC-responsive genes in quiescent cells (19). The repression of MYC´s transforming ability depends on the balance between the MYC-MAX and MXD/MNT/MGA-MAX complexes and the regulating members of the network exert their functions at different stages during the transition between proliferation and differentiation. Although distinct MYC-independent functions have been reported for some repressors, it seems that they could all behave in a very similar fashion. The exact significance of this putative functional redundancy in MYC repressors is unclear, but it seems that the differential timing of their induction ensures the regulation of the axis both during MYC overexpression and when MYC is subsequently downregulated. Taking into account the detrimental effect of the failure to control of MYC expression (i.e. by *MAX* mutations), the presence of various

regulators is likely to be an essential cellular mechanism in the inhibition of the transforming capacities of MYC through deregulation of the network.

The crucial role of MYC in cancer has formed the basis of numerous studies that have attempted to elucidate the involvement in tumorigenesis of the MXD1 family of transcriptional regulators. Thus, *MXI1* (MAX interactor 1) has been proposed as the candidate tumor suppressor gene that accounts for the 10q deletion frequently found in brain tumors (i.e. 80% of glioblastoma multiforme, and 15% of primary medulloblastoma). Other findings that are consistent with this include the identification of a mutation affecting *MXI1* in a medulloblastoma cell line, and that the reintroduction of MXI1 in a glioblastoma cell line lacking endogenous MXI1 resulted in a decreased growth rate and an accumulation of cells in the G2-M phase (20). These findings suggest that MXI1 may play a role in the development of other neural malignancies, as do MAX in PCC and MYCN in neuroblastoma. In addition, mice lacking MXI1 exhibit increased susceptibility to tumorigenesis (squamous cell carcinoma of the skin and malignant lymphoma) (21), and mutations in *MXI1* have also been reported in a small number of prostate tumors (22). Despite the enhanced ability of MXI1-deficient prostatic epithelium cells to proliferate, it seems that mutation of *MXI1* is a minor event in prostate cancer and therefore of unknown significance for prostate tumor development.

Of all the proposed transcriptional regulators of MYC, MNT is one of the most studied because its chromosomal location (17p13.3) is frequently deleted in cancer and, like MAX, it is ubiquitously expressed, with no fluctuations during the transition from G0-phase to S-phase. Although mice heterozygous for *MNT* mutations do not appear to be cancer prone, it has been demonstrated that MNT-deficient mouse embryonic fibroblasts and mammary glands exhibit many of the hallmark characteristics of cells that overexpress MYC (i.e. prone to apoptosis, efficiently avoid senescence and can be transformed with oncogenic RAS alone) (23). Nevertheless, studies focused on identifying *MNT*-inactivating mutations in tumors with common 17p losses (e.g. medulloblastoma) have found no abnormalities (24).

Therapeutic strategies targeting the MYC/MAX/MXD1 pathway

Site-directed mutagenesis experiments have consistently demonstrated the critical role of various aminoacids located within the bHLHZip domain of many components of the MYC/MAX/MXD1 network. Better knowledge of protein:protein and protein:DNA interactions could help to focus future therapeutic strategies on inhibitors of either the transforming activity of the complex or the recognition of target genes. Many compounds have already been tried because they interfere with MYC/MAX heterodimerization. However, given that MYC retains substantial MAX-independent activities, this may not be the best strategy for all tumour types (7). On the other hand, it has been clearly demonstrated that oncogene-induced tumorigenesis is reversible, and inactivation of a single oncogene within a primary tumor is sometimes sufficient to induce tumor regression. Thus, specific inactivation of MYC has been shown to reverse the malignant properties of various tumors (25, 26), and the combined inactivation of MYC and angiogenesis may be even more clinically effective (27). Another promising therapeutic approach is to block MYC-induced neoplasia by activating stress signaling pathways (28). Phosphorylation of MYC by the protein kinase PAK2 leads to degradation of MYC and therefore inhibition of proliferation and cell transformation. As previously mentioned, aberrant MYC expression is usually due to induction caused by upstream signals rather than mutations in the gene. Therefore the inhibition of MYC would tend to target the consequence more than the cause of oncogenesis (29). In addition, given that MYC is essential for the development of a broad range of adult organs, blocking MYC function may systemically trigger devastating and irreversible side-effects.

Oncogenes are more attractive therapeutic targets than tumor suppressor genes since it is easier to inhibit excessive activity than to restore lost activity in tumor cells. Nevertheless, strategies to restore protein function are viable anticancer therapeutic approaches nowadays (30), and may therefore be an option for *MAX*-related PCCs that have a potentially malignant phenotype. It is noteworthy that the transcriptional profile of MAX

tumors shows a significant enrichment of mTOR pathway components when compared to other mutated PCCs (data not shown). This finding is especially relevant since deregulation of both the mTOR pathway, and the upstream PTEN/PIK3CA/AKT1 axis, seems to be essential for the development of many PCCs (i.e. with mutations in *RET*, *NF1*, *TMEM127* or *MAX*) (Figure 1). Conditional PTEN knock-out mice present with metastatic bilateral PCCs (31), and RET-mediated cell-transforming capacity is critically dependent on the activation of the PIK3CA/AKT1 pathway (32). Furthermore, mTOR is constitutively activated both in NF1-deficient cells and in human tumors through the inactivation of tuberin by AKT (33). Finally, the PCC susceptibility gene *TMEM127* also functions by negatively regulating the mTOR pathway (34). Considering the transcriptional profile of MAX tumors, and since crosstalk between the PIK3CA/AKT1/mTOR and MYC/MAX/MXD1 pathways has been recently proposed (35), it is likely that mutations in *MAX* not only deregulate the MYC neoplastic switch, but also lead to the impairment of the mTOR pathway and PCC development. It has been reported that mTOR inhibitors (e.g. rapamycin) prevent proliferation of human neuroblastoma cells (36) and also inhibit the development of PCC in PTEN knock-out mice (37). In addition, it seems that sunitinib (recently shown to be a successful treatment for malignant PCCs) induces apoptosis in PC12 cells by inhibiting the VEGFR-2/AKT1/mTOR/S6K1 pathways. Since PC12 cells constitute a specific double-knockout model for MAX-related PCC, it seems plausible that targeting the mTOR pathway could be an effective therapy for malignant PCC patients carrying germline *MAX* mutations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This work was supported in part by the Fondo de Investigaciones Sanitarias (projects PS09/00942 and P1080883 to A.C. and M.R., respectively), and Mutua Madrileña (project AP2775/2008 to M.R.).

FIGURE LEGEND

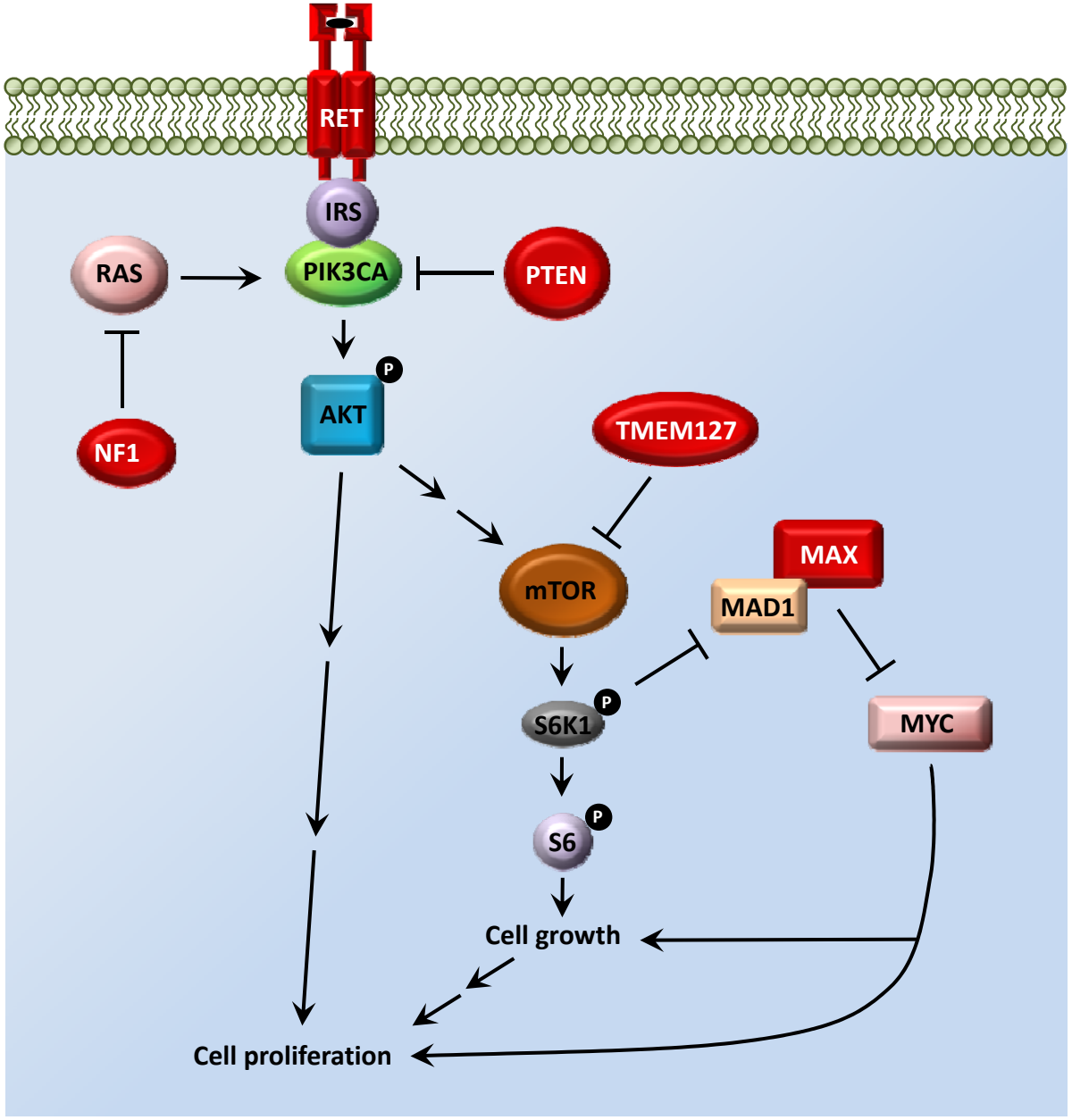
Figure 1. The PIK3CA/AKT1/mTOR pathway mediates downstream activation of genes involved in multiple cell processes such as regulation of cell growth, division, survival, and, when disrupted, tumorigenesis. In normal cells, upon binding to the glial-cell-line-derived neurotrophic factor (GDNF) family of ligands, the receptor tyrosine kinase RET triggers PIK3CA signaling via the recruitment of IRS proteins. PIK3CA subsequently activates (phosphorylates) AKT and this activated form of the protein regulates up to 100 downstream effector proteins involved in cell proliferation. In addition, the AKT-mediated phosphorylation of TSC2 indirectly leads to the activation of mTOR which regulates cell growth through phosphorylated S6K1. S6K1 also inhibits the tumor suppressor function of MAD1 and therefore leads to deregulation of MYC and also to cell grow and proliferation activation. Upstream, the pathway is negatively regulated by both PTEN, which directly antagonizes PIK3CA, and NF1, through RAS inhibition. TMEM127 is a negative regulator of mTOR and MAX represses MYC-dependent transcriptional activation.

References

1. Grandori C, Cowley SM, James LP, Eisenman RN. The Myc/Max/Mad network and the transcriptional control of cell behavior. Annu Rev Cell Dev Biol 2000; 16: 653-99.
2. Comino-Mendez I, Gracia-Aznarez FJ, Schiavi F, et al. Exome sequencing identifies MAX mutations as a cause of hereditary pheochromocytoma. Nat Genet 2011; 43: 663-7.
3. Cascon A, Pita G, Burnichon N, et al. Genetics of pheochromocytoma and paraganglioma in Spanish patients. J Clin Endocrinol Metab 2009; 94: 1701-5.
4. Lopez-Jimenez E, Gomez-Lopez G, Leandro-Garcia LJ, et al. Research resource: Transcriptional profiling reveals different pseudohypoxic signatures in SDHB and VHL-related pheochromocytomas. Mol Endocrinol 2010; 24: 2382-91.
5. Nair SK, Burley SK. X-ray structures of Myc-Max and Mad-Max recognizing DNA. Molecular bases of regulation by proto-oncogenic transcription factors. Cell 2003; 112: 193-205.
6. Ribon V, Leff T, Saltiel AR. c-Myc does not require max for transcriptional activity in PC-12 cells. Mol Cell Neurosci 1994; 5: 277-82.
7. Gallant P, Steiger D. Myc's secret life without Max. Cell Cycle 2009; 8: 3848-53.
8. Kohl NE, Kanda N, Schreck RR, et al. Transposition and amplification of oncogene-related sequences in human neuroblastomas. Cell 1983; 35: 359-67.
9. Seeger RC, Brodeur GM, Sather H, et al. Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. N Engl J Med 1985; 313: 1111-6.
10. Tang XX, Zhao H, Kung B, et al. The MYCN enigma: significance of MYCN expression in neuroblastoma. Cancer Res 2006; 66: 2826-33.
11. Thompson PM, Seifried BA, Kyemba SK, et al. Loss of heterozygosity for chromosome 14q in neuroblastoma. Med Pediatr Oncol 2001; 36: 28-31.
12. Volchenboum SL, Li C, Li S, et al. Comparison of primary neuroblastoma tumors and derivative early-passage cell lines using genome-wide single nucleotide polymorphism array analysis. Cancer Res 2009; 69: 4143-9.
13. Gattolliat CH, Thomas L, Ciafre SA, et al. Expression of miR-487b and miR-410 encoded by 14q32.31 locus is a prognostic marker in neuroblastoma. Br J Cancer 2011; 105: 1352-61.
14. Kelly K, Cochran BH, Stiles CD, Leder P. Cell-specific regulation of the c-myc gene by lymphocyte mitogens and platelet-derived growth factor. Cell 1983; 35: 603-10.
15. Evan GI, Wyllie AH, Gilbert CS, et al. Induction of apoptosis in fibroblasts by c-myc protein. Cell 1992; 69: 119-28.
16. Berg T, Cohen SB, Desharnais J, et al. Small-molecule antagonists of Myc/Max dimerization inhibit Myc-induced transformation of chicken embryo fibroblasts. Proc Natl Acad Sci U S A 2002; 99: 3830-5.
17. van Bokhoven H, Celli J, van Reeuwijk J, et al. MYCN haploinsufficiency is associated with reduced brain size and intestinal atresias in Feingold syndrome. Nat Genet 2005; 37: 465-7.
18. O'Hagan RC, Schreiber-Agus N, Chen K, et al. Gene-target recognition among members of the myc superfamily and implications for oncogenesis. Nat Genet 2000; 24: 113-9.
19. Ogawa H, Ishiguro K, Gaubatz S, Livingston DM, Nakatani Y. A complex with chromatin modifiers that occupies E2F- and Myc-responsive genes in G0 cells. Science 2002; 296: 1132-6.

20. Wechsler DS, Shelly CA, Petroff CA, Dang CV. MXI1, a putative tumor suppressor gene, suppresses growth of human glioblastoma cells. Cancer Res 1997; 57: 4905-12.
21. Schreiber-Agus N, Meng Y, Hoang T, et al. Role of Mxi1 in ageing organ systems and the regulation of normal and neoplastic growth. Nature 1998; 393: 483-7.
22. Eagle LR, Yin X, Brothman AR, Williams BJ, Atkin NB, Prochownik EV. Mutation of the MXI1 gene in prostate cancer. Nat Genet 1995; 9: 249-55.
23. Hurlin PJ, Huang J. The MAX-interacting transcription factor network. Semin Cancer Biol 2006; 16: 265-74.
24. Sommer A, Waha A, Tonn J, et al. Analysis of the Max-binding protein MNT in human medulloblastomas. Int J Cancer 1999; 82: 810-6.
25. Jain M, Arvanitis C, Chu K, et al. Sustained loss of a neoplastic phenotype by brief inactivation of MYC. Science 2002; 297: 102-4.
26. Shachaf CM, Kopelman AM, Arvanitis C, et al. MYC inactivation uncovers pluripotent differentiation and tumour dormancy in hepatocellular cancer. Nature 2004; 431: 1112-7.
27. Giuriato S, Ryeom S, Fan AC, et al. Sustained regression of tumors upon MYC inactivation requires p53 or thrombospondin-1 to reverse the angiogenic switch. Proc Natl Acad Sci U S A 2006; 103: 16266-71.
28. Huang Z, Traugh JA, Bishop JM. Negative control of the Myc protein by the stress-responsive kinase Pak2. Mol Cell Biol 2004; 24: 1582-94.
29. Soucek L, Whitfield J, Martins CP, et al. Modelling Myc inhibition as a cancer therapy. Nature 2008; 455: 679-83.
30. Ventura A, Kirsch DG, McLaughlin ME, et al. Restoration of p53 function leads to tumour regression in vivo. Nature 2007; 445: 661-5.
31. Korpershoek E, Loonen AJ, Corvers S, et al. Conditional Pten knock-out mice: a model for metastatic phaeochromocytoma. J Pathol 2009; 217: 597-604.
32. Segouffin-Cariou C, Billaud M. Transforming ability of MEN2A-RET requires activation of the phosphatidylinositol 3-kinase/AKT signaling pathway. J Biol Chem 2000; 275: 3568-76.
33. Johannessen CM, Reczek EE, James MF, Brems H, Legius E, Cichowski K. The NF1 tumor suppressor critically regulates TSC2 and mTOR. Proc Natl Acad Sci U S A 2005; 102: 8573-8.
34. Qin Y, Yao L, King EE, et al. Germline mutations in TMEM127 confer susceptibility to pheochromocytoma. Nat Genet 2010; 42: 229-33.
35. Zhu J, Blenis J, Yuan J. Activation of PI3K/Akt and MAPK pathways regulates Myc-mediated transcription by phosphorylating and promoting the degradation of Mad1. Proc Natl Acad Sci U S A 2008; 105: 6584-9.
36. Misawa A, Hosoi H, Tsuchiya K, Sugimoto T. Rapamycin inhibits proliferation of human neuroblastoma cells without suppression of MycN. Int J Cancer 2003; 104: 233-7.
37. Podsypanina K, Lee RT, Politis C, et al. An inhibitor of mTOR reduces neoplasia and normalizes p70/S6 kinase activity in Pten+/- mice. Proc Natl Acad Sci U S A 2001; 98: 10320-5.

Figure 1


RET
IRS
RAS
PIK3CA
PTEN
AKT
P
NF1
TMEM127
MAX
mTOR
MAD1
S6K1
P
MYC
S6
P
Cell growth
Cell proliferation