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Seminars in Cancer Biology 16 (2006) 265–274

seminars in
CANCER BIOLOGY

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Review

The MAX-interacting transcription factor network

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Abstract

The small bHLHZip protein MAX functions at the center of a transcription factor network that governs many aspects of cell behavior, including cell proliferation and tumorigenesis. MAX serves as a cofactor for DNA binding by the various members of this network, which include the MYC family of oncoproteins and a group of putative MYC antagonists that include MNT, MXD1-4 (formerly MAD1, MXI1, MAD3 and MAD4) and MGA. The many heterodimerization partners of MAX raises questions concerning the dynamics of MAX interactions and the functional consequences of the switching of Max partners. Here we review the activities of MAX, its interaction partners, and recent results showing that tissues lacking the MAX-interacting protein MNT are predisposed to tumor formation.

Keywords: MAX; MYC; MNT; Mad; MXD

Contents

1. Introduction

Amplification of *MYC* family genes (c-*MYC*, N-*MYC* and L-*MYC*) is one of the most frequent events associated with human cancer. *MYC* genes are also a relatively common target of chromosomal translocations associated with different types of cancer, especially hematopoietic malignancies. These tumor-related events are linked to the disruption of the stringent cis-acting regulatory control systems that normally keep MYC gene transcription and MYC protein levels at relatively low levels in most cells. In addition, enhanced and/or deregulated MYC expression is often found in tumor cells in the absence of any identified physical alterations at the *MYC* gene level. In such cases, elevated MYC levels appear to be due to defects in various signal transduction pathways known to play key roles in the regulation of cell proliferation. This is not surprising since nearly all known mitogenic pathways ultimately stimulate *MYC* gene expression (reviewed in Ref. [1]).

So why do so many mitogenic, as well as anti-mitogenic, signal transduction pathways converge on *MYC* regulation? It has been long known that MYC is capable of driving quiescent cells into the cell cycle, blocking exit from the cell cycle and blocking terminal differentiation of many cell types. Studies of mice lacking c-MYC and N-MYC suggest that these activities of MYC proteins normally serve in the development and function of virtually all tissues and organ systems [2–8]. The essential roles played by MYC proteins during embryonic development and their frequent involvement in tumorigenesis can be explained, in part, by their direct transcriptional regulation of a number of genes whose encoded products play key roles in cell cycle

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doi:10.1016/j.semcancer.2006.07.009

control. In addition, MYC has been implicated in the direct regulation of a diverse set of genes encoding proteins associated with protein translation and metabolism (reviewed in Refs. [9,10]). Studies examining the MYC transcriptome also reinforced the notion that MYC stimulates a large number of genes encoding proteins that participate in anabolic pathways, including ribosome biogenesis [11–16]. The ability of MYC to regulate such a broad swath of genes – either directly or indirectly – has recently been reconciled and expounded on by studies showing that MYC proteins govern ribosome biogenesis directly by regulating RNA polymerases I and III [17–20]. The involvement of MYC in regulating all three RNA eukaryotic polymerases and their roles in regulating ribosome biogenesis and cell growth (accumulation of cell mass) have been the subject of several recent reviews [21–23].

Consistent with MYC's involvement in regulating all three RNA polymerases and it's effect on global gene expression, experiments examining c-MYC bound to chromatin [24–26] find MYC binding (either through direct or indirect mechanisms) to a large number of sites. Recent estimations suggest that there are around 25,000 sites in the human genome where c-MYC may bind. Although many of the binding sites identified are situated nearby protein coding genes, many are also associated with sequences that probably give rise to RNA species, such as inhibitory micro RNAs (miRNAs) [26], that serve other functions. Indeed, a cluster of miRNAs transcribed by c-MYC was recently shown to modulate the expression of the E2F1 transcription factor [27]. E2F1 in turn plays a role in mediating MYC activities in cell proliferation and apoptosis [28].

In addition to the far-reaching effects MYC has on cell growth and proliferation, c-MYC can also facilitate the delivery of nutrients and metabolites to cells by stimulating blood vessel formation [29,30]. This combination of activities further explains the frequent association of elevated MYC levels with oncogenesis, as well as the mid-gestation lethality caused by removal of c-*MYC* or N-*MYC*. But cells are not completely defenseless to the cancer-promoting effects of MYC, as forced expression of MYC also sensitizes many cell types to apoptosis (reviewed in Refs. [31,32]). Sensitivity to apoptosis seems to be triggered, at least in part, by direct regulation of pro-apoptotic genes by MYC, but is exacerbated when growth factor signaling systems that promote cell survival are deficient. The pro-apoptotic activity of MYC is further revealed by many studies showing that secondary events that disrupt pro-apoptotic genes and pathways play a critical role in MYC-dependent tumorigenesis [31,32]. Therefore, human tumors that exhibit deregulated and/or abnormally high levels of MYC probably have either lost their apoptotic response to MYC or are not programmed to respond in this manner.

2. MYC and MAX

A number of functional domains have been identified in MYC family proteins that are required for, or contribute to their oncogenic activity. These include the amino (N)-terminal regions known as MYC Box 1 (MB1), and MB2 (reviewed in Ref. [23]). The MB1 and MB2 regions are involved in the recruitment of various coactivator proteins with activities that include histone acetylation and histone remodeling (Fig. 1, and reviewed in Ref. [23]). These activities are thought to promote transcription by relaxing local chromatin structures and facilitating the loading of the basal transcriptional machinery. MB1 also contains closely spaced serine and threonine residues that mediate phosphorylation-dependent regulation of MYC degradation [33,23]. These same residues also influence the apoptotic and tumorigenic activities of MYC [34].

In addition to MB1 and MB2, the basic-helix-loop-helix-leucine zipper (bHLHZip) motif located at the extreme C-terminus of MYC proteins is critical to their function as transcription factors and their activities in cell proliferation and transformation [35–37]. The bHLHZip motif is found in a number of different transcription factors and functions as a contiguous DNA binding (specified primarily by a basic region) and dimerization module (specified largely by the HLHZip). Dimerization in this class of factors is required for specific DNA binding, and multiple examples exist for both bHLHZip-mediated homodimerization and heterodimerization being utilized for this purpose. In the case of MYC family proteins, they appear to not homodimerize under physiological conditions, but instead heterodimerize with the small bHLHZip protein MAX [38–40].

MAX is a ubiquitously expressed phosphoprotein with two commonly expressed isoforms that migrate as 21 and 22 kDa proteins [40]. The p21 form differs from the p22 form by the differential splicing of a sequence encoding nine amino acids in the N-terminus of MAX. There is evidence that these MAX isoforms have differential DNA binding and unique transcription and biological activities ([41–43], see more below). Several other smaller MAX isoforms, presumably generated by differential splicing, are sometimes observed ([44], P.J. Hurlin, unpublished), but it remains unclear what functions these isoforms may have.

Based on in vitro site-selection assays, the MYC-MAX complex binds DNA at so-called E-box sequences (CANNTG). However, studies of MYC binding sites in vivo suggest that the DNA binding specificity of MYC-MAX complexes is broader and context dependent [45]. It is interesting to note that variations of the E-box are the preferred binding site of a large number of transcription factors that contain either the bHLHZip motif or the related bHLH motif. While some proteins within these families, like MyoD, appear to have a more restricted collection of target genes, perhaps numbering in the hundreds [46], others like MYC family proteins appear to have thousands of target genes as discussed above. It is not clear what determines the number of direct target genes a given bHLH or bHLHZip binds to, but it is probably related to the specific transcription cofactors they recruit and the chromatin modifying activities that the cofactors possess. For MYC-MAX complexes, their extensive repertoire of co-activators and associated chromatin modifying activities [33] likely provide a great deal of flexibility (and complexity) in target gene access and regulation. Moreover, some MYC transcriptional cofactors may be limiting in cells, such that large increases in MYC protein levels, as is found in many tumors and during cell cycle entry, may cause the composition of cofactors bound to MYC to change and alter their target gene