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B-Myb, Cancer, Senescence, and MicroRNAs

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Abstract

The transcription factor B-Myb plays a critical role in regulating gene expression and is implicated in controlling carcinogenesis and cellular senescence. Transcription of the *B-Myb* gene is regulated by retinoblastoma proteins acting directly on the *B-Myb* promoter. Recently, we found that microRNAs also control the abundance of *B-Myb* mRNA during senescence, adding another level of complexity to *B-Myb* regulation. This review focuses on the importance of B-Myb in cancer and senescence, with an emphasis on the regulation of B-Myb expression and activity.

Introduction

B-Myb (v-Myb myeloblastosis viral oncogene homolog [avian]-like 2 [MYBL2]) encodes a transcription factor that regulates the expression of numerous genes during cell cycle progression (1). The Myb gene family of transcription factors is present in all vertebrates (2). *c-Myb* is the homologue of the *v-Myb* oncogene, which is present in avian retroviruses that cause acute leukemia (3, 4). The other two family members, *A-Myb* and *B-Myb,* were cloned based on homology to *c-Myb* (5). In mammals, *c-Myb* and *A-Myb* expression is restricted to specific cell types and stages of development, whereas *B-Myb* is expressed in virtually all proliferating cells (2, 5). In cultured cells, *B-Myb* expression is highest in the S phase of the cell cycle, and changes in *B-Myb* expression have been linked to growth arrest, apoptosis, carcinogenesis, and senescence (6, 7). Thus, it is essential to determine the mechanisms that regulate expression of this important gene.

B-Myb regulates genes important in cell proliferation and survival

All three Myb proteins bind to consensus Myb-binding sites (MBS) in DNA (2), but the phenotypes of knockout mice that lack individual Myb family members are strikingly different, with mice lacking *B-Myb* dying very early in development because the blastocyst inner cell mass does not form (8). These diverse phenotypes demonstrate that the Myb proteins carryout different biological functions. Divergent function of the Myb proteins was also suggested by expression profiling, which revealed that ectopic expression of each of the three Myb proteins activates different sets of genes (9).

B-Myb stimulates transcription of genes that promote entry into the S and M phases of the cell cycle (1, 6). For several of these genes, such as DNA topoisomerase IIα and c-Myc, B-Myb acts by binding directly to MBSs in their promoters {*e.g.*, (10, 11)}. B-Myb activates the expression of other genes that lack MBSs, including *B-Myb* itself, by interacting with

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transcription factors that bind to the promoters of these genes (12, 13). In conjunction with E2F proteins, B-Myb also stimulates the expression of genes required for the G2/M phase of the cell cycle (14). In addition, binding of B-Myb to the multiprotein LINC/DREAM complex regulates the ability of this complex to affect gene expression (15-18). B-Myb can also negatively repress gene expression, perhaps by competing with other transcription factors whose binding sites overlap with MBSs in target promoters {*e.g.*, (19, 20)}. Finally, B-Myb associates directly with clathrin and filamin, components of the mitotic spindle (21). The absence of B-Myb reduces the amount of clathrin in the spindle and causes mitotic arrest.

The transcriptional activity of B-Myb is regulated by post-translational modifications and by interactions with other proteins (1). B-Myb is activated by phosphorylation and acetylation, which release it from transcriptional co-repressors (22, 23). However, phosphorylation also leads to ubiquitylation of the B-Myb protein (24), decreasing its half-life and limiting its activity to S phase. B-Myb activity is also regulated by binding to transcriptional co-activators, co-repressors, and other proteins {reviewed in (6)}.

B-Myb in cancer and senescence

Given the provenance of *c-Myb* as a cellular proto-oncogene and the ability of B-Myb to regulate the expression of cell cycle genes, it is not surprising that B-Myb is involved in cell proliferation and carcinogenesis. *B-Myb* expression is required for entry into S phase and can overcome growth inhibitory signals (25-27). Cytogenetic analysis of several types of cancers revealed amplification of chromosome 20q13, where *B-Myb* is located {*e.g.*, (28)}. In addition, *B-Myb* over-expression occurs in several cancers and has been linked to aggressive tumor growth and poor outcomes in neuroblastomas and other tumors {e.g., (28-30)}. Conversely, *B-Myb* repression can inhibit the proliferation of normal and tumor cells (11, 26, 31, 32). The ability of B-Myb to increase the expression of anti-apoptotic genes such as Bcl2, survivin, and clusterin may also contribute to cancer progression (17, 33-35). Interestingly, certain inherited sequence variants of *B-Myb* are associated with altered cancer risk (30, 36).

Cellular senescence, a form of irreversible growth arrest, appears to be an important obstacle that cells must bypass during carcinogenesis (37). Because *B-Myb* expression is strongly repressed during senescence, it seems reasonable that loss of B-Myb expression may play an important role in senescence. Several findings support this hypothesis. Repression of *B-Myb* inhibits proliferation of mouse BALB/c3T3 fibroblasts, whereas constitutive *B-Myb* expression allows the cells to grow with reduced growth factors (32). In primary mouse embryonic fibroblasts, over-expression of the *ras* oncogene induces premature senescence, but co-expression of *B-Myb* abrogates this response (20). Our group previously showed that inhibition of *B-Myb* expression by shRNAs induces senescence in primary human fibroblasts and HeLa cervical cancer cells (38). In human embryonic lung fibroblasts, B-Myb is a direct transcriptional repressor of the cyclin-dependent kinase inhibitor $p16^{INK4a}$, which is involved in the induction of senescence (39). Over-expression of *B-Myb* in these cells increases their *in vitro* lifespan, and *B-Myb* repression induces premature senescence. Taken together, these data suggest that B-Myb plays a central role in controlling senescence.

B-Myb is regulated at the post-transcriptional level by microRNAs during senescence

B-Myb expression is low in quiescent cells because of Rb-mediated repression (7). Repressive complexes between members of the E2F family and the Rb family (specifically E2F4/p107 and E2F4/p130) bind to E2F sites in the *B-Myb* promoter in G0 cells and repress

its activity (40). Recently, our group discovered that *B-Myb* is also repressed at the post-transcriptional level by microRNAs (41).

Small non-coding microRNAs regulate gene expression by base-pairing with specific mRNA targets and affecting their translation or stability. We found that the expression of approximately 50 cellular microRNAs changes in HeLa cells undergoing Rb-induced senescence. Several members of the miR-29 and miR-30 families are up-regulated in senescent HeLa cells and primary human foreskin fibroblasts. This up-regulation is Rb-dependent and likely to involve the oncogene *c-Myc*. It has been shown that c-Myc binds directly to the miR-29 and miR-30 promoters and acts as a transcriptional repressor of these genes (42). However, c-Myc itself is an E2F-responsive gene repressed by the Rb-pathway during senescence (43). Taken together, these data suggest that miR-29 and miR-30 up-regulation during senescence is due to Rb-driven inhibition of *c-Myc* expression and the consequent loss of c-Myc-mediated repression of the promoters controlling transcription of these microRNAs.

By using reporter constructs, mutational analysis of microRNA binding sites, and ectopic expression or inhibition of members of the miR-29 and miR-30 families in both cell types, we demonstrated that these microRNAs bind to the 3′UTR of *B-Myb* mRNA during senescence and reduce the amount of this mRNA, presumably by reducing its stability. Furthermore, overexpression of miR-29 and miR-30 inhibits the expression of endogenous *B-Myb* and reduces DNA synthesis. The inhibition of DNA synthesis by these microRNAs was partially rescued by exogenous expression of *B-Myb*, suggesting that *B-Myb* repression is responsible, at least in part, for growth inhibition in cells over-expressing miR-29 and miR-30. Finally, antagonizing the activity of miR-29 and miR-30 allows a population of cells to escape Rb-induced senescence, demonstrating the importance of miR-29 and miR-30 in this process.

These experiments demonstrate the complexity of *B-Myb* regulation during Rb-induced senescence (Figure 1). Activation of the Rb pathway mobilizes p107 and p130 which, in complex with E2F4, bind to the *B-Myb* promoter and inhibit transcription. In addition, Rb activation increases the expression of miR-29 and miR-30, which bind to *B-Myb* mRNA and destabilize it. As noted above, B-Myb is also regulated at the protein level by post-translational modification and association with other cellular proteins. Presumably, this three-tiered regulation of *B-Myb* evolved to ensure profound inhibition of B-Myb activity and complete blockade of cell cycle progression.

miR-29 and miR-30 affect the expression of numerous genes in addition to *B-Myb*, and many of these targets may also play a role in senescence or carcinogenesis. For example, Croce and colleagues have identified DNA methyltransferase 3A and 3B as targets of miR-29 (44). Similarly, in addition to miR-29 and miR-30, numerous microRNAs are up-regulated or down-regulated during senescence (41). These microRNAs may also regulate the expression of genes involved in senescence.

Conclusions and perspectives

B-Myb exerts powerful effects on cell behavior. The multitude of B-Myb protein partners and target genes allows it to regulate many important cellular processes including proliferation, senescence, apoptosis, and mitosis. Given this spectrum of activities and its association with human cancer, it may be possible to exploit B-Myb as an important prognostic, diagnostic and therapeutic tool. Do mutations that affect the binding of Rb proteins to the *B-Myb* promoter or the binding of microRNAs to *B-Myb* mRNA affect cancer risk by regulating levels of B-Myb? Is the presence of such mutations or the level of B-Myb

itself an informative biomarker? Is it possible to influence the activity of B-Myb and effect cellular behavior by modulating its expression or its ability to bind to DNA or its protein partners, including components of the LINC/DREAM complex and the mitotic spindle? Are there as-yet-unrecognized layers of complexity in the regulation of B-Myb that can be exploited therapeutically? The central role played by B-Myb in cellular proliferation and related processes suggests that the factors that regulate the expression and activity of B-Myb deserve special scrutiny in attempts to develop rational approaches to control cancer.

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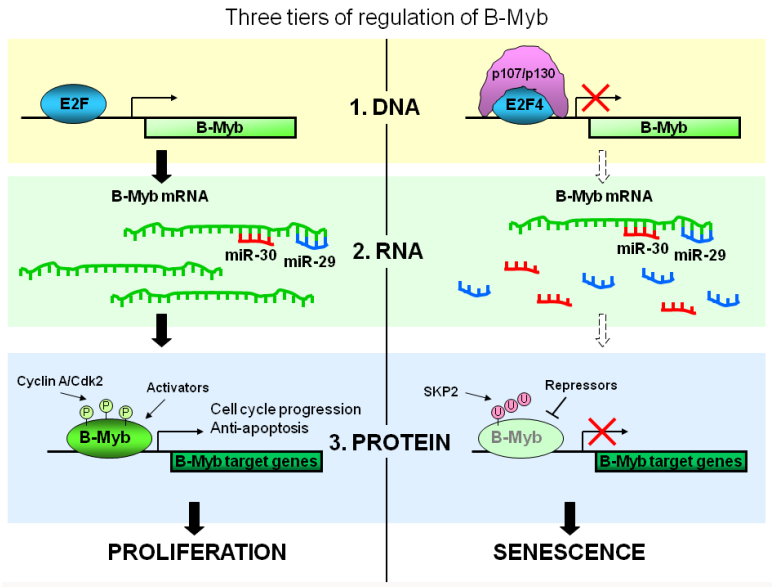

Figure 1. Three-tiered control of B-Myb

Activation of the Rb pathway during senescence restricts B-sMyb action at multiple levels. At the DNA level, activating E2F complexes at the B-Myb promoter are replaced by inhibitory complexes between E2F4 and p107 and p130. At the RNA level, elevated levels of miR-29 and miR-30 base-pair to the 3′UTR of B-Myb mRNA, reducing its abundance. At the protein level, reduced expression of B-Myb-responsive genes lowers cyclin/cdk activity and the extent of activating phosphorylation of B-Myb. In addition, ubiquitylation and degradation of B-Myb, as well as preferential association with co-repressors, may further inhibit its ability to activate target genes.