The great MYC escape in tumorigenesis

Increased wild-type *MYC* expression occurs frequently in human cancers, except in Burkitt's lymphoma, where the translocated *MYC* allele is frequently mutated at several hotspots, including a major one at threonine-58. Acute *MYC* expression increases p53 or ARF levels and induces apoptosis, and previous transgenic animal studies revealed frequent inactivating mutations of p53 or p19ARF in transgenic Myc-induced lymphomas. Lowe and coworkers (Hemann et al., 2005) demonstrate that wild-type *MYC* can also trigger apoptosis by inducing Bim, which neutralizes Bcl-2. In contrast, the *MYC* point mutants failed to induce Bim, promoting murine lymphomas that escaped both wild-type p53 and p19ARF, and in doing so, evaded apoptosis.

The proto-oncogene *Myc* was first discovered as the cellular homolog of v-*myc*, which was retrovirally captured from avian genomes and mutated (Sheiness and Bishop, 1979). When reintroduced back into host cells, v-*myc* acutely promoted cancers in various avian tissues. The discovery of human *MYC* as a target for activation by chromosomal translocation in Burkitt's lymphoma first identified it as a prototypical oncogene that proved later to be commonly involved in many human cancers (Dalla-Favera et al., 1982). In contrast to activation of wild-type *MYC* in solid tumors, *MYC* activation in Burkitt's lymphoma is mediated via its juxtaposition with immunoglobulin enhancers. This predisposes the translocated *MYC* allele to mutations due to the somatic hypermutation machinery that is required for antibody diversity. Two studies with large numbers of human Burkitt's lymphoma samples mapped several *MYC* mutational hotspots, which occurred in about 20% of cases, with a major one centering around T58 (cited in Hoang et al., 1995). These mutations have increased transforming activity in vitro, and some mutations are associated with diminished Myc's apoptotic potential. Intriguingly, the mechanism by which these mutations enhance transformation in vivo was unknown until the recent study, which utilized a murine model of lymphomagenesis (Hemann et al., 2005).

The Myc protein consists of over 430 amino acids with 150 amino-terminal residues comprising the transactivation domain that encompasses two conserved Myc family protein boxes I and II. Box I contains the conserved T58 residue (Adhikary and Eilers, 2005). The carboxy-terminal 90 amino acids comprise the DNA and dimerization domain that binds an obligate partner protein, Max. Clues that missense mutations in the Myc transactivation domain may be significant arose from recurrent mutations of the residue analogous to human T58 and the surrounding region in different, distinct isolates of the v-*myc* oncogene (Papas and Lautenberger, 1985). Although mutations affecting T58 and the surrounding area augment Myc's transforming potential, it was unclear whether this increased transforming potential was due to increased cell proliferation or decreased apoptosis (Henriksson et al., 1993). In fact, several studies suggest that the inhibitory effect of p107 on Myc activity may be abrogated by these mutations (Hoang et al., 1995). Furthermore, it was shown that phosphorylation of T58 leads to the proteasomal degradation of Myc, and hence mutation of T58 could cause an elevation of Myc levels that contribute to tumorigenesis (Salghetti et al., 1999). It is also notable that phosphorylation of T58 can be enhanced by activated Ras, thereby linking T58 to cooperativity between Myc and Ras in transformation (Sears et al., 1999). While these hypotheses are tractable and plausible, there has been a dearth of genetic evidence to support these mechanisms. In vivo models to test the transforming activities of Myc mutants were lacking until the study by Hemann et al. (2005).

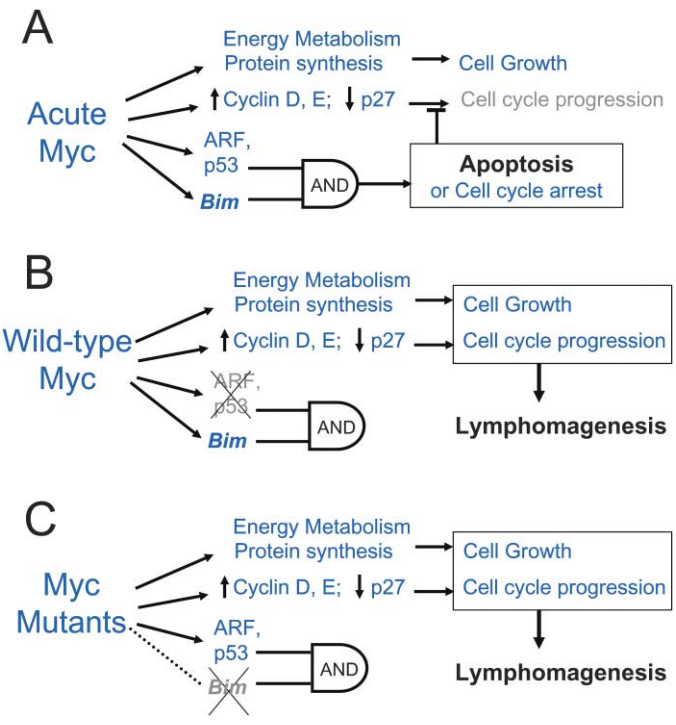

Figure 1. Mechanisms for escaping MYC-induced apoptosis

A: Acute or ectopic activation of Myc induces target genes involved in cell growth and cell cycle progression, but the activation of ARF, p53, and Bim (which inhibits Bcl-2) results in apoptosis or cell cycle arrest. The "AND" logic gate depicts the requirement of at least two signals, ARF-p53 AND Bim, to trigger apoptosis.

B: Wild-type Myc induces lymphomagenesis coordinately with the inactivation of ARF or p53.

C: Myc mutants derived from Burkitt's lymphoma fail to activate Bim and hence promote lymphomagenesis despite the presence of wild-type p53 or ARF.

The authors sought to determine the in vivo transforming activities of wild-type *MYC* as compared with Burkitt's lymphoma-derived *MYC* mutants T58A and P57S (Hemann et al., 2005). T58 is a target for GSK3β phosphorylation that is dependent on the proline residue at position 57 and prior phosphorylation of S62; hence, mutation of P57 also

abrogates T58 phosphorylation (Hoang et al., 1995). Irradiated recipient mice were transplanted with murine fetal liver-derived hematopoietic progenitors that express retroviral vectors that harbor wild-type or mutant MYC transgenes. Animals transplanted with hematopoietic cells containing *MYC* mutants rapidly succumbed to lymphomas, whereas those receiving cells with wild-type *MYC* had a delayed onset of lymphomas. Retrovirally transduced cells with wild-type or *MYC* point mutants demonstrated similar proliferative potential as determined by BrdU uptake; however, apoptosis was significantly diminished with cells expressing *MYC* mutants. The authors examined specifically the expression of p19ARF and p53 in the mutant *MYC*-induced tumors, since it was previously demonstrated that these checkpoint genes are frequently mutated in *MYC*-induced lymphomas (Eischen et al., 1999). Unlike the wild-type *MYC* tumors, the mutant *MYC* tumors had wild-type p19ARF and p53, but the expression of Bim was markedly diminished. To test the significance of the inability of Myc mutants to induce Bim, which binds to and inactivates Bcl-2, the authors measured the ability of wild-type and mutant Myc to induce apoptosis in wild-type and knockout Bcl-2 murine embryo fibroblasts (MEFs). In wild-type Bcl-2 cells, wt Myc induced higher levels of apoptosis than the Myc mutants. However, in Bcl-2 null MEFs, Myc mutants induce a similar level of apoptosis as wild-type Myc. These observations support the hypothesis that diminished activation of Bim by Myc mutants causes a decrease in Myc-mediated apoptosis.

Additional experiments using $Bim^{-/-}$ stem cells revealed that wild-type and Myc mutants develop lymphomas at similar rates. These observations suggest that the inability of Myc mutants to induce Bim contributes to their enhanced tumorigenicity. However, the mechanism by which Myc activates Bim was not defined in this study.

To determine the clinical significance of their findings, the authors demonstrate a differential level of Bim expression in human Burkitt's lymphomas bearing wild-type versus mutant *MYC* alleles. Indeed, Bim expression was virtually absent in lymphomas with *MYC* Box I mutations. The authors also noted from the literature that Burkitt's lymphomas with p53 mutations appear devoid of coding sequence mutations in *MYC*. These correlations support the key observation in this study that Burkitt's lymphoma-derived *MYC* Box I mutations increase oncogenicity by diminishing the level of apoptosis through the failure to activate Bim.

The ability of endogenous Myc to stimulate cell proliferation and of ectopic, pathologic Myc levels to transform cells is linked to their target transcriptomes (Adhikary and Eilers, 2005). The expression of endogenous MYC is tightly regulated, such that its expression is highly sensitive to external cellular cues, as well as internal homeostatic mechanisms. In contrast, ectopic Myc expression not only affects the physiological Myc-responsive transcriptome, but also acutely induces a set of genes that respond to pathophysiologic levels of Myc. It stands to reason that Myc should be tightly regulated, but mechanisms to keep cells with deregulated Myc in check must also evolve to protect the host organism from sustaining lethal oncogenic events. As such, acute activation of Myc in normal cells triggers cell growth arrest or apoptosis through the induction of p53 or p19ARF (Eischen et al., 1999). Hemann et al. demonstrate that in addition to these downstream events, wild-type, but not mutant, Myc induces Bim to trigger apoptosis (Figure 1), confirming.a previous report (Egle et al., 2004). Their study further shows that mutant Myc could induce lymphomagenesis in the presence of wild-type p53 and p19ARF, suggesting that Myc can trigger apoptosis through multiple independent mechanisms. Furthermore, their observations suggest that these independent pathways must be integrated and a cumulative threshold effect reached to trigger apoptosis. Hence, mutations of p53 or p19ARF collaborating with wild-type Myc, or mutations of Myc that fail to induce Bim, appear sufficient for initiating lymphomagenesis.

It should be noted that in addition to the suppression of Myc-induced apoptosis by Myc Box I mutations, Myc must also activate genes that are required for tumorigenesis (Adhikary and Eilers, 2005). The spectrum of genes induced by Myc involves almost all known cellular functions, with overrepresentation of genes that regulate the cell cycle and carbohydrate and nucleotide metabolism, as well as ribosomal and mitochondrial biogenesis. Although Lowe and coworkers attributed higher transforming potencies of Myc mutants in vivo to diminished apoptosis, they did not directly assess whether other tumorigenic events such as adaptation to the tumor microenvironment or angiogenesis could be affected by Myc Box I mutations. Nonetheless, their demonstration of a differential effect between wild-type and mutant Myc suggests that alterations in apoptosis play a central role in the activity of Myc mutants.

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