Molecular & Cellular Oncology 2:2, e975083; January 1, 2015; Published with license by Taylor & Francis Group, LLC

Bim's up first

Auto-commentary on (Bim is the primary mediator of Myc-induced apoptosis) in multiple solid tissues. http://dx.doi.org/10.1016/j.celrep.2014.07.057

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Keywords: Myc, Apoptosis, Bim, p19Arf, Rosa26-MycER^{T2}

In vivo analysis of the genetic determinants of Myc-induced apoptosis reveals a specific requirement for the Bcl2 family protein Bim (Bcl2l11). Surprisingly, apoptosis induced by Myc in multiple solid tissues does not require p19Arf (Cdkn2a), whereas Puma (Bbc3) is required only in the context of sensitization by Myc to death induced by DNA damage.

MYC is one of the most frequently overexpressed oncogenes across a spectrum of human cancers and a growing body of evidence suggests that MYC may serve as an obligate conduit of oncogenic signaling, even in the absence of overt *MYC* amplification.[1,2] It is textbook knowledge that the induction of mitochondrial apoptosis by MYC serves to limit the oncogenic potential of this proto-oncogene, yet the obvious therapeutic potential implied by this remains largely untapped. This may be in part due to the widely-held belief that MYC-induced apoptosis strictly requires an intact CDKN2A^{ARF}/MDM2/TP53 pathway, which is itself abrogated in the vast majority of human cancers. In light of reports from several groups that MYC can induce apoptosis independently of this pathway, we sought to re-examine the genetic requirements for Myc-induced apoptosis, exploiting the unique features of the Rosa26-MycER^{T2} mouse line that employs a tamoxifen-inducible fusion protein comprised of human MYC and a modified ligand-binding domain of the estrogen receptor to achieve acute deregulation of near-physiological levels of Myc simultaneously in multiple adult tissues.[3] Acute systemic activation of MycER^{T2} in this model drives ectopic proliferation in most adult tissues but apoptosis is restricted to the intestine, where MycER^{T2} expression is highest. Activation of MycER^{T2} does, however, elicit pro-apoptotic signaling in tissues other than the intestine, as evidenced by the sensitization of such tissues to doxorubicin-induced cell death. We showed that under both circumstances (apoptosis induced by high levels of Myc alone and sensitization to an additional pro-apoptotic signal by lower levels of Myc) apoptosis occurs unabated in the absence of p19Arf (encoded by Cdkn2a) but is suppressed by deletion of *Bcl2l11*, which encodes the proapoptotic protein Bim.[4] Our results are closely mirrored by those from an independent group examining MYC-dependent apoptosis in human tumor cell lines in response to bortezamib,[5] effectively ruling out a species-specific or system-specific requirement for Bim.

Bim is one of several proapoptotic Bcl2-Homology domain 3 (BH3)-only proteins (others include Bbc3/Puma, Pmaip1/Noxa, p22Bid, and Bad) that function by binding to antiapoptotic Bcl2-homologous (BH) proteins, including Bcl2 itself, Bcl2l1 (BclXL), Mcl1, and Bcl2a1a (A1). Sequestration of these antiapoptotic proteins permits oligomerization of the effector BH family proteins Bax and Bak, resulting in pore formation and thereby permeabilization of the mitochondrial outer membrane, effectively demarcating a point of no return in the apoptotic cascade. Whether or not a cell dies in response to proapoptotic signaling is thus critically dependent upon the relative levels of pro- and anti-apoptotic BH family proteins.[6] One might then expect that loss of any one BH3-only protein would have much the same effect as loss of any other; however, this is not the case. We showed that Myc-induced apoptosis in the intestine requires Bim but not Puma and, conversely, that apoptosis induced in the intestine by the DNA-damaging agent doxorubicin requires Puma but not Bim; apoptosis induced by the combination of both requires both Bim and Puma. Thus, distinct BH3-only proteins mobilize in response to distinct death signals, yet can combine to overcome antiapoptotic buffering.

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Submitted: 09/19/2014; Revised: 09/20/2014; Accepted: 09/22/2014
http://dx.doi.org/10.4161/23723556.2014.975083

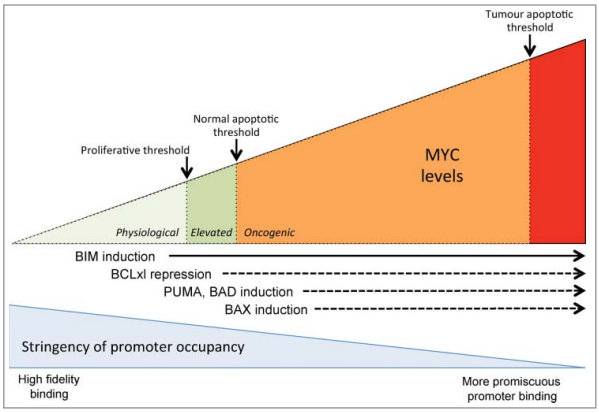

Figure 1. Model for engagement of Bcl2-homologous genes by rising Myc levels. Our data indicate that, unlike other Bcl2 homologous (BH) family genes, the *Bcl2l11* locus (encoding Bim) is bound by physiological levels of Myc. As Myc levels rise, so does expression of Bim, and a threshold level of Bim is required to overcome the physiological apoptotic threshold. In cancer, the apoptotic threshold is set much higher, requiring a stronger apoptotic signal to trigger cell death. Oncogenic levels of Myc may contribute to stronger apoptotic signaling by engaging low stringency elements in the promoters of multiple BH family genes. The precise sequence in which this might occur remains to be resolved.

A Special Relationship Between Myc and Bim

Chromatin immunoprecipitation analysis revealed binding of endogenous Myc to the *Bcl2l11* locus in untransformed mouse embryo fibroblasts cultured in 10% serum. Importantly, promoter occupancy was not saturated by endogenous Myc, as activation of MycER^{T2} resulted in increased binding. Similar binding kinetics were observed at the *BCL2l11* locus in non-transformed MCF10A human epithelial cells. Strikingly, in these cells no MYC binding, endogenous or inducible, was observed at other BH family genes, including *BCL2*, *BCLX*, *BBC3* (encoding PUMA), *PMAIP1* (encoding NOXA), *BID*, *BAD*, *BAX*, or *BAK*. This contrasts with promoter occupancy of BH family genes in tumor cells derived from a genetically engineered mouse model of pancreatic cancer;[7] such cells express very high levels of Myc and exhibit Myc binding to all of the above promoters except for *Noxa* and *Bak*. Although this difference might be explained by any number of factors, from tissue-specific chromatin configurations to differences between species, in light of recent reports studying promoter occupancy by different levels of Myc,[8,9] a very simple model emerges. We suggest that the *Bcl2l11* (Bim) promoter contains high-affinity Myc binding sites that are bound at lower (i.e., physiological or somewhat elevated) levels of Myc, whereas other BH family genes contain lower affinity binding sites and thus require higher levels of Myc for binding (**Fig. 1**). Induction of Bim by physiological levels of Myc would not automatically drive apoptosis because a threshold level of Bim induction is required to alone overcome anti-apoptotic buffering.[4] Such cells would nonetheless be "primed" to die in the presence of another pro-apoptotic signal or sub-optimal survival signaling. A striking example of this is the requirement for Bim during Tgfβ1-induced apoptosis in Apc-deleted intestinal epithelium that expresses elevated levels of Myc due to deregulated Ctnnb1 activity.[10] This model has 2 clear implications: (1) higher levels of Myc elicit a stronger proapoptotic signal by engaging more BH family genes; and (2) the requirement for Bim can be overridden at very high levels of Myc.

Tumor cells evolve continuously to cope with the challenges of relentless oncogenic signaling and survival in a hostile milieu. However, their adaptation is imperfect and rather like a series of stopgap measures adopted under extreme duress. Strategies to exploit this maladaption may lead to improved therapeutic response rates. Augmenting intrinsic prodeath signals, for instance through the use of BH3 mimetics to overcome antiapoptotic buffering, thus holds great promise for tumors expressing high levels of MYC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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