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

Daniel J. Murphy* and Nathiya Muthalagu
Institute of Cancer Sciences; University of Glasgow and the CRUK Beatson Institute for Cancer Research; Glasgow, UK

Keywords: Myc, Apoptosis, Bim, p19Arf, Rosa26-MycERT2

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/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-MycERT2 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 MycERT2 in this model drives ectopic proliferation in most adult tissues but apoptosis is restricted to the intestine, where MycERT2 expression is highest. Activation of MycERT2 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 bortezomib,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.

© Daniel J. Murphy and Nathiya Muthalagu
*Correspondence to: Daniel J. Murphy; Email: daniel.murphy@glasgow.ac.uk
Submitted: 09/19/2014; Revised: 09/20/2014; Accepted: 09/22/2014
http://dx.doi.org/10.4161/23723556.2014.975083
This is an Open Access article distributed under the terms of the Creative Commons Attribution-Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The moral rights of the named author(s) have been asserted.

www.landesbioscience.com Molecular & Cellular Oncology e975083-1
A Special Relationship Between Myc and Bim

Chromatin immunoprecipitation analysis revealed binding of endogenous Myc to the Bcl2l11 locus in untransformed mouse embryonic fibroblasts cultured in 10% serum. Importantly, promoter occupancy was not saturated by endogenous Myc, as activation of MycER\textsuperscript{1,2} 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\textsubscript{L}, BCC3 (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,\textsuperscript{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,\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 overruled 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 stop-gap measures adopted under extreme duress. Strategies to exploit this maladaptation may lead to improved therapeutic response rates. Augmenting intrinsic pro-death 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.

References

1. Meyer N, Penn LZ. Reflecting on 25 years with MYC. Nat Rev Cancer 2008; 8:976-90; PMID:19029958; http://dx.doi.org/10.1038/nrc2231
2. Morton JP, Sansom OJ. MYC-y mice: from tumour initiation to therapeutic targeting of endogenous MYC. Molec Oncol 2013; 7:248-58; PMID:23523308; http://dx.doi.org/10.1016/j.molonc.2013.02.015
3. Murphy DJ, Juntila MR, Pouyet L, Karnezis A, Shchors K, Bui DA, Brown-Swigart L, Johnson L, Evan GI. Distinct thresholds govern Myc's biological output in vivo. Cancer Cell 2008; 14:447-57; PMID:18961836; http://dx.doi.org/10.1016/j.ccr.2008.10.018
4. Muthalapaz N, Juntila MR, Wiese KE, Wolf E, Morton J, Bauer B, Evan GI, Eders M, Murphy DJ. BIM is the primary mediator of MYC-induced apoptosis in multiple solid tissues. Cell Rep 2014; 8:1347-53; PMID:25176652; http://dx.doi.org/10.1016/j.celrep.2014.07.057
5. Wirf M, Stojeanovic N, Christian J, Paul MC, Stauber RH, Schmid RM, Hacker G, Kramer OH, Saur D, Schneider G. MYC and EGR1 synergize to trigger tumor cell death by controlling NOXA and BIM transcription upon treatment with the proteasome inhibitor bortezomib. Nucleic Acids Res 2014; 42:10433-47; PMID:25147211; http://dx.doi.org/10.1093/nar/gku763
6. Tait SW, Green DR. Mitochondrial regulation of cell death. Cold Spring Harbor Perspect Biol 2013; 5: a008706; PMID:24003207
7. Morton JP, Timpson P, Karim SA, Ridgway RA, Ahineos D, Boyle B, Jamieson NB, Oen KA, Lowy AM, Brunton VG, et al. Mutant p53 drives metastasis and overcomes growth arrest/senescence in pancreatic cancer. Proc Natl Acad Sci U S A 2010; 107:246-51; PMID:20018721; http://dx.doi.org/10.1073/pnas.0908428107
8. Saho A, Kress TR, Pelizzola M, de Pretis S, Gorski MM, Teu A, Morelli MJ, Bera P, Doni M, Verrecchia A, et al. Selective transcriptional regulation by Myc in cellular growth control and lymphomagenesis. Nature 2014; 511:488-92; PMID:25043028; http://dx.doi.org/10.1038/nature13537
9. Wala S, Lorenzin F, Morton J, Wiese KE, von Eys B, Herold S, Rycal L, Dumay-Odolot H, Karim S, Barrakhm M, et al. Activation and repression by oncogenic MYC shape tumour-specific gene expression profiles. Nature 2014; 511:483-7; PMID:25043018; http://dx.doi.org/10.1038/nature13473
10. Wiener Z, Band AM, Kallio P, Hogstrom J, Hyvonen V, Kajälainen S, Ritosu O, Haglund C, Kruuna O, Robine S, et al. Oncogenic mutations in intestinal adenomas regulate Bim-mediated apoptosis induced by TGF-β. Proc Natl Acad Sci U S A 2014; 111:E2229-36; PMID:24825889; http://dx.doi.org/10.1073/pnas.1404644111