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Loss of BIM augments resistance of ATM-deficient thymocytes to DNA damage-induced apoptosis but does not accelerate lymphoma development

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To the Editor,

Malignant transformation proceeds as nascent neoplastic cells accumulate oncogenic lesions that confer the 'hallmarks of cancer', including self-sufficiency for growth signalling and resistance to anti-growth signals, such as cell senescence and apoptosis.[1,2]

Exposure to DNA damaging agents can facilitate neoplastic transformation in mice[3] and humans.[4,5] The transcription factor p53 plays a critical role in preventing tumorigenesis, including lymphoma development driven by γ-radiation.[6–8] Apoptosis induction was thought to underpin p53-mediated tumour suppression, however recent findings using various gene targeted mice demonstrated that apoptosis induction (as well as cell cycle arrest and senescence) are dispensable for p53-mediated tumour suppression.[9–11] These findings raise the question: can potentially oncogenic DNA lesions induce apoptosis through p53-independent tumour suppression pathways?

We described a role for BIM as a critical inducer of apoptosis (independently of p53) to prevent transformation of T-cell progenitors drive by oncogenic lesions arising from T-cell receptor gene rearrangement.[12] To examine whether this BIM-mediated apoptotic pathway might be relevant in the context of tumorigenesis driven by DNA double-strand breaks more broadly, we took advantage of ATM-deficient mice, a model of the human syndrome ataxia telangiectasia, characterised by a high propensity for tumour development.[13] This disease is caused by loss of ATM, a DNA damage sensing kinase, acting at the site of DNA lesions to initiate the DNA damage response.[14] Interestingly lymphoma development in this model has also been shown to be RAG1/2-dependent.[15]

We found that thymocytes deficient for ATM are relatively insensitive to cell death induced by γ-irradiation or etoposide, as reported,[16] with the additional loss of BIM conferring additional protection (Figure 1a). Loss of either ATM or BIM conferred resistance to cisplatin; while as expected[17,18] only loss of BIM conferred resistance to the glucocorticoid dexamethasone (Figure 1a).

Loss of ATM resulted in reduced circulating T cells,[13] and this could be rescued by concomitant loss of BIM (Supplementary Figure 1). As reported,[17] loss of BIM resulted in elevated blood T-cell numbers. Loss of neither BIM nor ATM altered erythrocyte

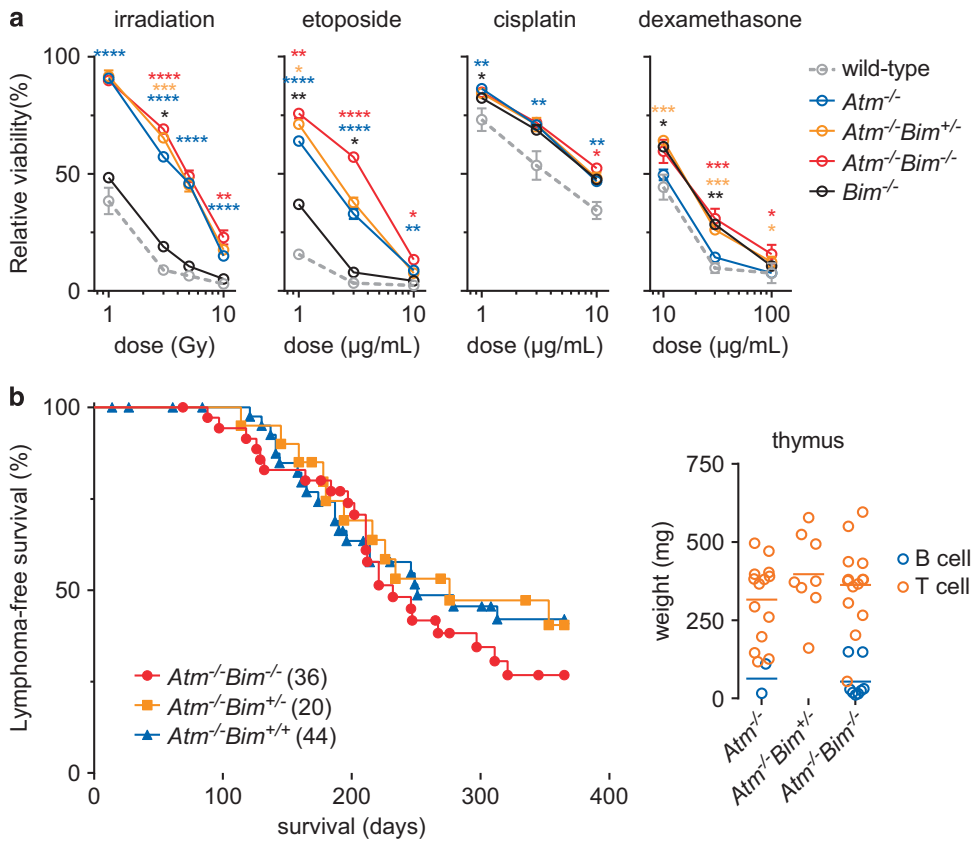

Figure 1 Loss of BIM augments resistance of $Atm^{-/-}$ thymocytes to DNA damage-induced apoptosis, but does not impact lymphoma development. (**a**) Thymocytes survival was determined following treatment with the indicated agents relative to untreated cells at 24 h. $n = 3$–9. (**b**) Mice were reconstituted with HSPCs and lymphoma-free survival was determined (median survival: $Atm^{-/-}Bim^{-/-}$ 232d, $Atm^{-/-}Bim^{+/-}$ 276d and $Atm^{-/-}Bim^{+/+}$ 251d). Thymus weight from the lymphoma-bearing animals and tumour immunophenotype was determined (donor origin was confirmed with congenic CD45 staining). $n = 8$–22; tumour type mean shown. (**a**) $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$; black (wild-type vs $Bim^{-/-}$), blue (wild-type vs $Atm^{-/-}$), yellow ($Atm^{-/-}$ vs $Atm^{-/-}Bim^{+/-}$) and red ($Atm^{-/-}$ vs $Atm^{-/-}Bim^{-/-}$). Mean ± S.E.M. depicted

numbers, while loss of ATM resulted in an elevation of platelet numbers, which was normalised by concomitant loss of BIM. ATM-deficiency reduced thymus cellularity and concomitant loss of BIM afforded partial rescue (Supplementary Figure 1).

To determine the impact of compound loss of BIM and ATM on lymphoma development, a haematopoietic reconstitution approach was adopted to obviate potentially confounding effects caused by ATM loss in other tissues.[13] Thus, lethally irradiated wild-type mice were reconstituted with bone marrow from $Atm^{-/-}Bim^{-/-}$, $Atm^{-/-}Bim^{+/-}$ or $Atm^{-/-}Bim^{+/+}$ mice, but no differences in the onset, rate or severity of lymphoma development were observed between the different genotypes (CD45 congenic markers were used to confirm donor origin of all lymphomas; Figure 1b).

We conclude that while loss of BIM confers additional resistance to DNA damage-induced apoptosis and can ameliorate certain haematopoietic defects caused by loss of ATM, it does not significantly impact lymphoma development in this disease setting.

Conflict of Interest

The authors declare no conflict of interest.

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