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

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Dear 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 (TCR) 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 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.

Competing Interest

The authors declare no conflict of interest.

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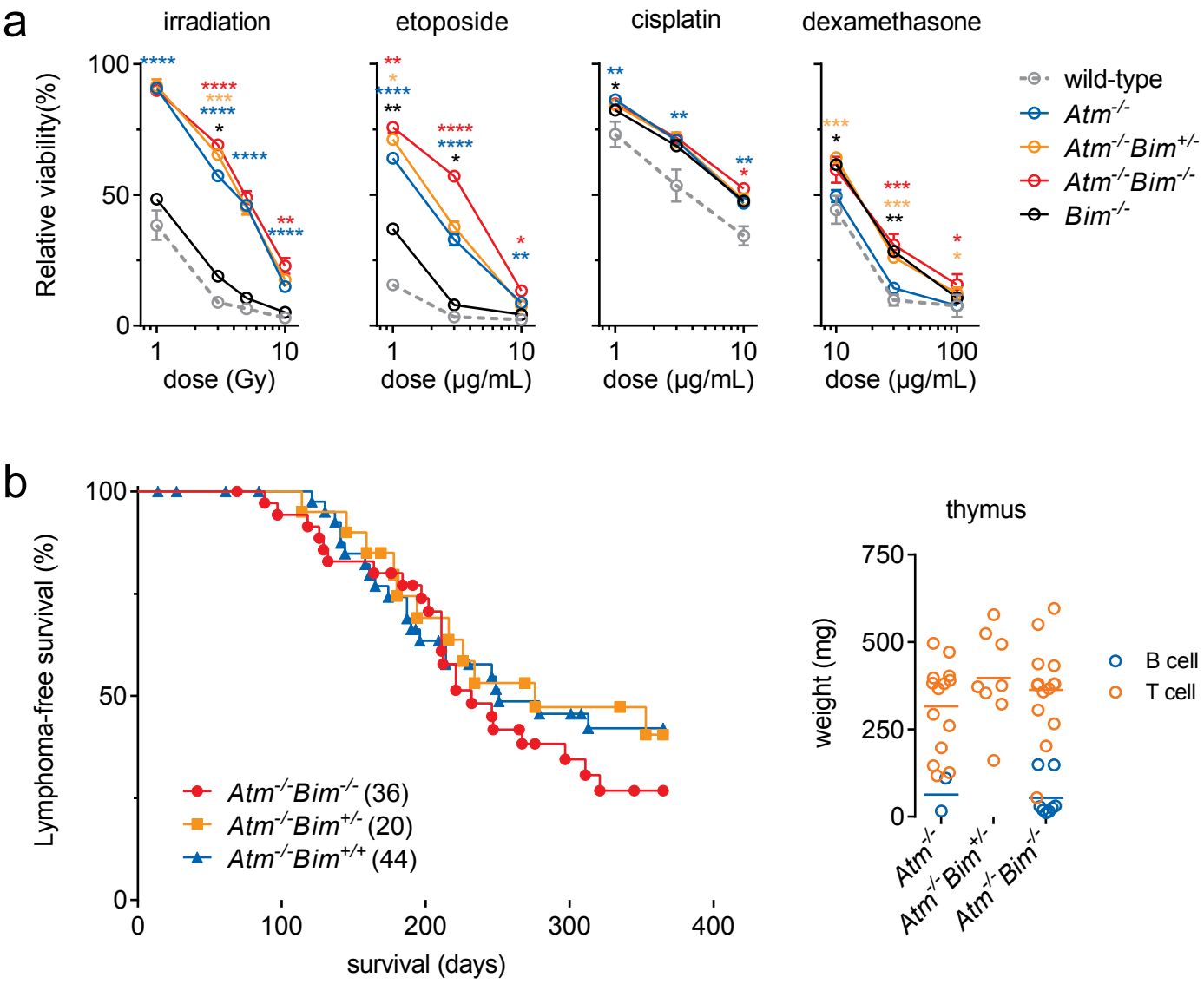
References

1. Hanahan D, et al. Cell. 2011;144(5):646-74.
2. Delbridge AR, et al. Cold Spring Harbor perspectives in biology. 2012;4(11).
3. Kaplan HS. Natl Cancer Inst Monogr. 1964;14:207-20.
4. Richardson D, et al. Radiation research. 2009;172(3):368-82.
5. Krestinina L, et al. Radiation and environmental biophysics. 2010;49(2):195-201.
6. Kemp CJ, et al. Nat Genet. 1994;8:66-9.
7. Donehower LA, et al. Nature. 1992;356:215-21.
8. Jacks T, et al. Curr Biol. 1994;4(1):1-7.

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*$^{+/-}$], red [*Atm*$^{-/-}$ vs *Atm*$^{-/-}$*Bim*$^{-/-}$]. mean +/- SEM depicted.


a
irradiation
etoposide
cisplatin
dexamethasone
Relative viability(%)
100
50
0
1
10
100
dose (Gy)
dose (µg/mL)
wild-type
Atm-/-
Atm-/-Bim+/-
Atm-/-Bim-/-
Bim-/-
b
Lymphoma-free survival (%)
survival (days)
0
100
200
300
400
Atm-/-Bim-/- (36)
Atm-/-Bim+/- (20)
Atm-/-Bim+/+ (44)
thymus
weight (mg)
750
500
250
B cell
T cell
Atm-/-
Atm-/-Bim+/-
Atm-/-Bim-/-

Delbridge *et. al.* Supplementary Information

Supplementary Figure 1. Loss of BIM rescues T cell numbers in the blood and thymus of ATM-deficient mice. Erythrocyte, platelet, and T cell numbers were determined in the blood and cellularity in the thymus of 8 wk old mice of the indicated genotypes. n = 3-12. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. mean +/- SEM depicted.

Supplementary Methods

Mice

All experiments with mice were conducted according to the guidelines of The Walter and Eliza Hall Institute of Medical Research Animal Ethics Committee. *Bim*$^{-/-}$ [17] and *Atm*$^{-/-}$ mice [13] have been previously described. All mice were crossed onto a C57BL/6 background for at least 10 generations before commencement of our studies.

Reconstitution studies were performed as described in [19] and lymphoma-free survival was determined by Kaplan-Meier analysis.

Survival assay

Thymocytes were treated *in vitro* with either γ-irradiation from a ^{60}Co source (Best Theratronics, Kanata, ON, Canada), etoposide (Toposar, Pfizer, New York City, NY, USA), cisplatin (1134357-100MG, Sigma Aldrich; St. Louis, MO, USA) or dexamethasone (D4902-25MG, Sigma Aldrich; St. Louis, MO, USA) and survival was determined by flow cytometry relative to untreated cells at 24 h based on lack of staining with AnnexinV and propidium iodide (2μg/mL P3566, Thermo Fisher Scientific; Waltham, MA, USA).

Blood and thymic analysis

Blood counts were determined using automated blood analysis (ADVIA 2120 analyzer; Siemens Healthcare PTY, Ltd, Bayswater, VIC, Australia) or by with cell counting (CasyCell Counter; Schaefe System GmbH, Neunkirchen, Germany).

T cell subsets and tumour immunophenotype was determined by flow cytometry using the following antibodies CD4 [YTA3.2.1], CD8 [YTS169], B220 [RA3-6B2], CD45.1 [A201] and

CD45.2 [5.450.15.2], conjugated to FITC, PE or APC (prepared in-house). 1-3 x 10^6 cells were stained and analysed on FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA).

Statistical analysis

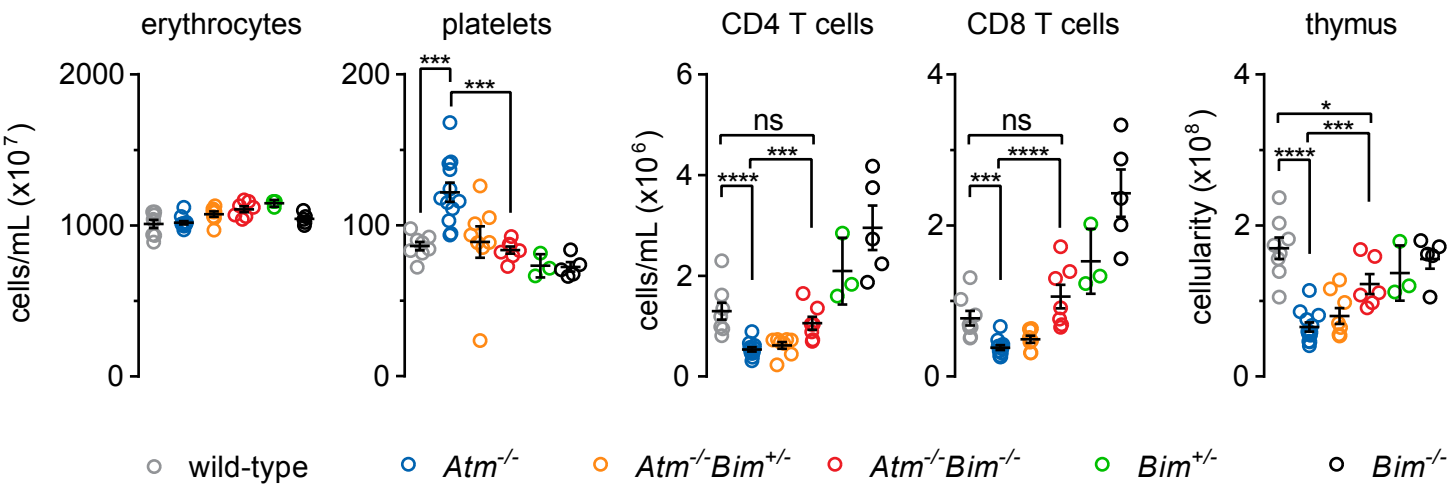
Statistical analysis was performed in GraphPad Prism (GraphPad Software Inc, La Jolla, CA, USA) using Student t test (unpaired, equal variance).

Author contributions

This study was conceived by AD with guidance from AS. Experiments were designed, conducted and analysed by AD, SG and AS. Manuscript was prepared by AD and AS with assistance from SG.

Supplementary References

9. Brady CA*, et al. Cell* 2011 May 13; **145**(4)**:** 571-583.
10. Li T*, et al. Cell* 2012 Jun 8; **149**(6)**:** 1269-1283.
11. Valente LJ*, et al. Cell reports* 2013 May 30; **3**(5)**:** 1339-1345.
12. Delbridge AR*, et al. The Journal of experimental medicine* 2016 Sep 19; **213**(10)**:** 2039-2048.
13. Barlow C*, et al. Cell* 1996; **86**(1)**:** 159-171.
14. Lavin MF*, et al. Biomolecules* 2015 Oct 23; **5**(4)**:** 2877-2902.
15. Liao M-J*, et al. Genes Dev* 1999; **13**(10)**:** 1246-1250.
16. Biswas S*, et al. Cell Death Differ* 2013 Jul; **20**(7)**:** 869-877.
17. Bouillet P*, et al. Science* 1999; **286**(5445)**:** 1735-1738.
18. Erlacher M*, et al. Blood* 2005 Aug 23; **106**(3)**:** 4131-4138.
19. Delbridge AR*, et al. Blood* 2015 May 21; **125**(21)**:** 3273-3280.


erythrocytes
platelets
CD4 T cells
CD8 T cells
thymus
cells/mL (x10^7)
cells/mL (x10^6)
cellularity (x10^8)
2000
1000
0
200
100
6
4
2
4
2
***
ns
****
*
wild-type
Atm-/-
Atm-/-Bim+/-
Atm-/-Bim-/-
Bim+/-
Bim-/-