Complex downstream effects of nuclear export inhibition in B-cell lymphomas: a possible role for activation-induced cytidine deaminase (AID)

We read with great interest the recent paper by Azmi *et al.*[1] who reported the *in vitro* and *in vivo* anti-lymphoma activity of novel specific inhibitors of nuclear export (SINE). The authors focused on targeting a main nuclear export protein called chromosome maintenance region 1 (CRM1) or exportin-1 (XPO1) because it had been reported to be a major player in drug resistance in many tumor types in which it might be over-expressed. A major obstacle for targeting XPO1 is the fact that it is responsible for the nuclear export of many proteins harboring canonical or non-canonical nuclear export signals. In their study, however, Azmi *et al.*[1] claimed their new agent KPT-185 and its derivatives act highly specifically through covalent binding to the Cys-528 residue of XPO1. The authors also provided strong evidence that the observed activity was due to the blocking of the XPO1-mediated nuclear export of p53 and p73, which allowed their nuclear accumulation resulting in tumor suppression.

We, however, would like to propose an additional mechanistic explanation for the observed pro-apoptotic effect when CRM1 is targeted in B-cell lymphomas. We propose that CRM1 inhibition in B-cell lymphomas affects the subcellular distribution of activation-induced cytidine deaminase (AID). AID is an essential enzyme for secondary immunoglobulin gene diversification through class switch recombination and somatic hypermutation by triggering DNA breaks.[2] It is well-known that AID shuttles between the nucleus and cytoplasm to perform its functions, which is proposed to be one of the mechanisms for the tight regulation of its DNA damaging activity.[3] Furthermore, AID has a well-defined nuclear export signal (NES) located at its C-terminus and shuttles in a CRM1-dependent manner.[4] Although AID mistargeting can cause oncogenic DNA lesions, which could lead to lymphomas,[5] several studies suggested that increased levels of AID expression can trigger apoptotic cell death because of the higher levels of genotoxic stress.[6,7] We have also shown previously that the increased resident time of AID in the nucleus can cause cell death in a Burkitt's lymphoma cell

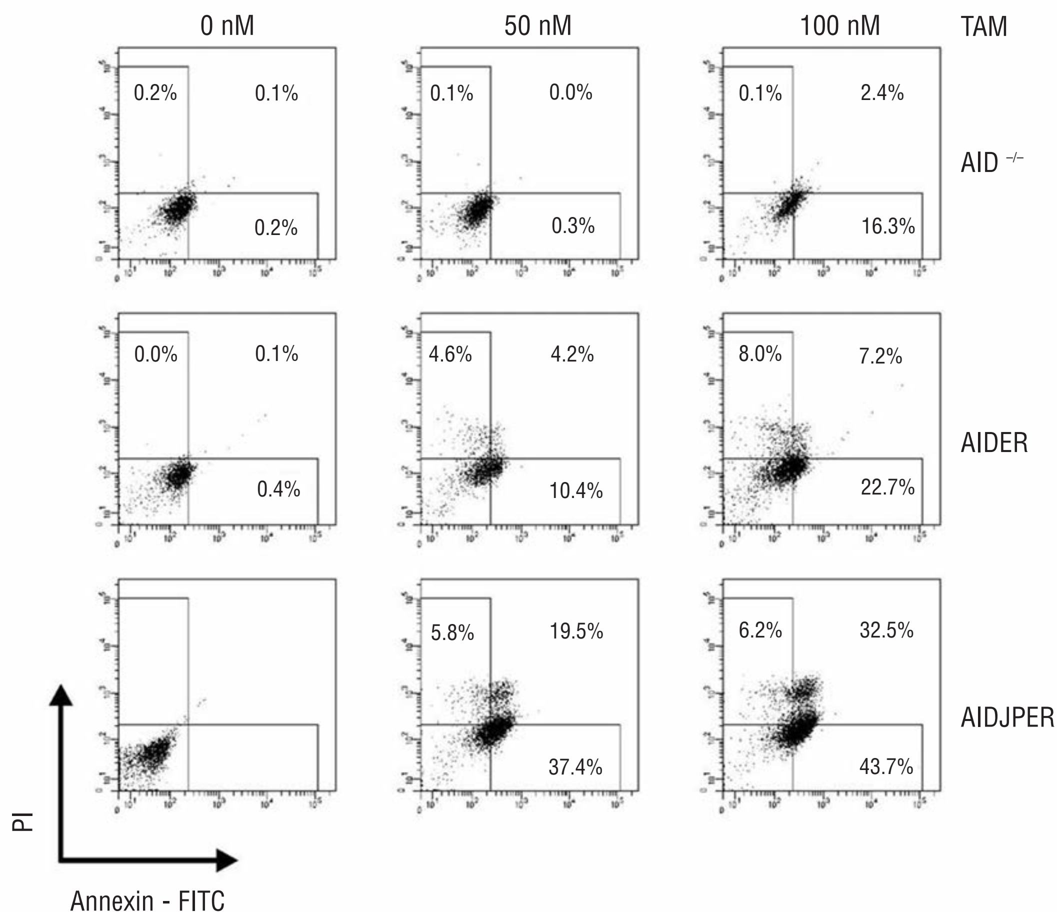

Figure 1. Induction of apoptosis in BL2 AID$^{-/-}$ cell lines. AID$^{-/-}$ BL2 (AID$^{-/-}$), AID(wt)ER+BL2 (AIDER) and JP8BdelER+BL2 (AIDJPER) cell lines (at $1x10^6$/mL) were cultured for 24 h with increasing concentrations of OHT (TAM) ranging from 0 to 100 nM. The percentage of viable and apoptotic cells was assessed on LSRII flow cytometer after standard Annexin-V and propidium iodide (PI) staining. Further details are given in the text.

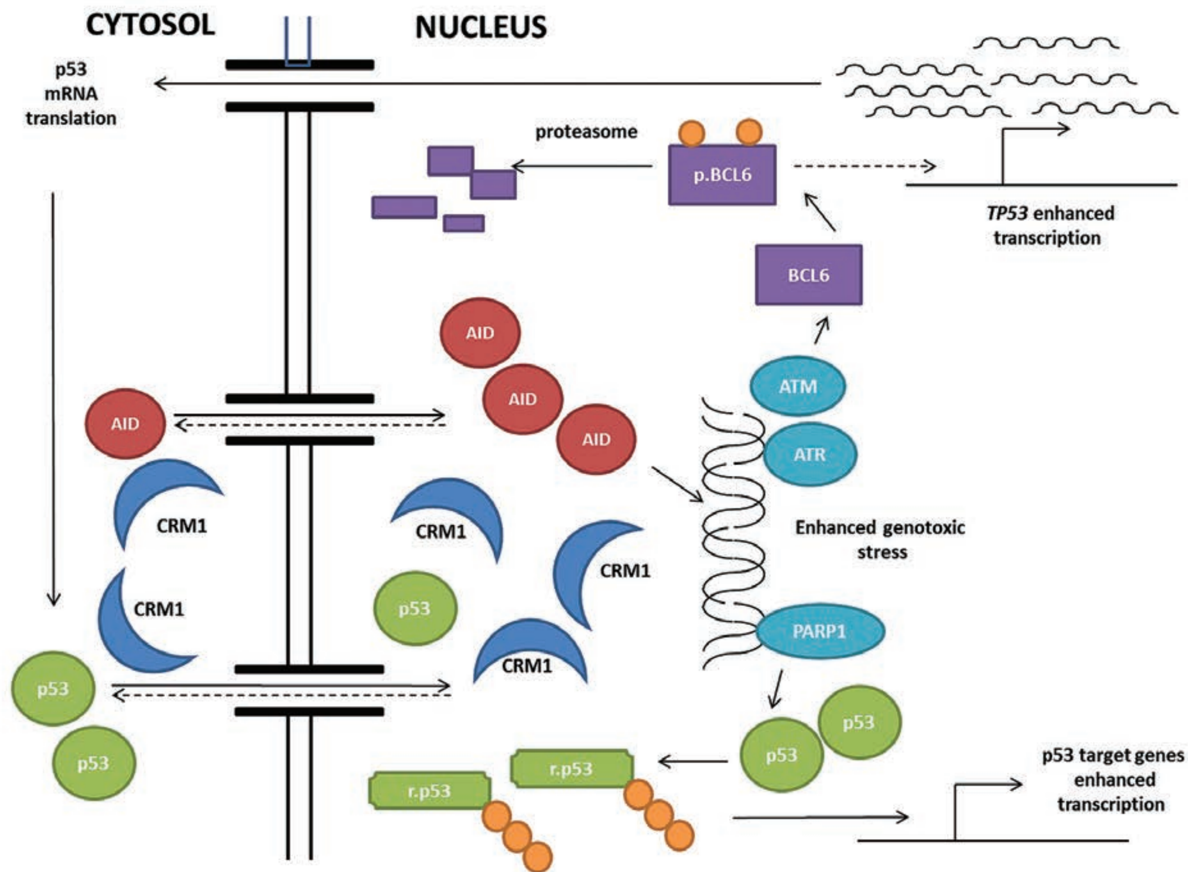

Figure 2. Schematic model representing the pathways leading to p53 induction after SINEs treatment of B-cell lymphomas. Dotted lines represent decreased nuclear export of AID and p53 after treatment with SINEs. Further explanations are given in the text. AID: activation-induced cytidine deaminase; ATM: ataxia telangiectasia mutated protein, serine/threonine kinase; Ataxia telangiectasia and Rad3 related protein; PARP1: Poly [ADP-ribose] polymerase 1; BCL6: B-cell lymphoma 6 protein; CRM1: chromosome maintenance region 1; r.: ribosylation; p.: phosphorylation,

line (BL2) both *in vitro* and *in vivo*.[8] We controlled subcellular AID distribution by using AID$^{-/-}$ BL2 cell lines stably transfected with the AID(wt)ER or the AID(Jp8Bdel)ER transgenes. This system allows a strict control of AID translocation to the nucleus by the addition of tamoxifen (OHT). In our system, the AID(wt)ER protein could be normally exported from the nucleus whereas AID(Jp8Bdel)ER could not as this mutant is lacking the C-terminal NES. As shown in Figure 1, OHT addition triggered a dose-dependent apoptosis in AID(wt)ER or the AID(Jp8Bdel)ER cell lines, which was more pronounced in the case of AID(Jp8Bdel)ER. These findings lead us to propose that, along with the effect on p53 and p73, the SINEs developed by Azmi *et al.*[1] could exert their effect on germinal center (GC) derived B-cell lymphomas through an increase of the nuclear AID, i.e. through conferring higher levels of genotoxic stress. Indeed, in their report, Azmi *et al.*[1] carried out extensive studies of two GC-derived cell lines (WSU-FSCCL and WSU-DLCL2) and GC-derived B-cell lymphomas usually express easily detectable levels of AID.[9] Although AID expression in the SINE-treated WSU-FSCCL and WS-DLCL2 cell lines has not been tested, the fact that a p53 knockdown could not completely rescue cell viability of SINE-treated cells supports the presence of additional mechanisms. Also, the authors did not observe any cytotoxic effect in peripheral B cells that generally express neither p53 nor AID.

Actually, the blockade of the nuclear export of AID can indirectly cause further p53 accumulation by at least two additional mechanisms. Firstly, increased AID can cause a higher level of single stranded DNA breaks that is sensed by PARP proteins, which in turn can cause p53 ribosylation.[10] Ribosylated p53 does not readily interact with CRM1 and that leads to its enhanced nuclear accumulation and enhanced activity.[10] Secondly, in germinal center B cells, the AID-induced genotoxic stress can cause ATM/ATR-dependent BCL6 phosphorylation and subsequent degradation.[11] As BCL6 acts as transcriptional inhibitor of the *TP53* gene, its degradation activates *TP53* transcription.[12] *TP53* mRNA is further translated and this causes an overall increase in the p53 expression in the cell. This model is schematically summarized in Figure 2 and demonstrates the complex interdependent pathways that might be affected through CRM1 inhibition in GC B cells.

Finally, we conclude that SINE administration in GC-lymphomas is one of the approaches to therapeutically target AID and enhance its activity, which in turn can cause enhanced genotoxic stress and trigger apoptotic cell death. Furthermore, the complexity of the effect of nuclear export inhibition in GC-derived B-cell lymphomas suggests a

rationale for combination of SINE with other novel therapeutic approaches under investigation, such as BCL6 inhibitors[13] and RAD51 inhibitors.[14]

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