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

We read an enlightening piece of work from Shivarov and colleagues on the downstream secondary effects of CRM1 inhibition that involves activation-induced (cytidine) deaminase (AID).[1] These Authors have previously shown evidence that nuclear retention of AID plays a strong role in promoting apoptosis in Burkitt's lymphomas.[2] They have also performed site directed mutations/truncation experiments in which the absence of nuclear exclusion sequence results in AID nuclear retention is concurrent with apoptotic cell death.[2] These mechanistic insights have led them to propose that, in addition to p53 and p73, the role of AID nuclear retention cannot be ignored when defining the mechanism of CRM1 inhibitor interactions (especially in lymphoma models). We also welcome their important and logical suggestions of rationally selecting combinations with Bcl6 and Rad51 inhibitors.

Adding to their interesting findings, we would like to mention that CRM1 is recognized as the major nuclear exporter of most NES carrying nuclear proteins that are more than 40KDa in size. This was recently demonstrated by Thakar and colleagues using mass spectroscopy that show CRM1 having approximately 200 target cargo proteins that are shuttled out of the nucleus through NES recognition.[3,4] Moreover, targeted inhibition of CRM1 should result in global re-organization of NES containing proteins (including p53 family members, AID and others) in cancer cells as well as normal cells. We recently proposed that a successful evaluation of such global rearrangements requires more holistic approaches, such as computational biology and mathematical modeling; these studies are currently underway in our laboratory.[5] Adding to the complexity, CRM1 is also recognized as an exporter of pre-cursor microRNAs (originally, Xpo5 was considered the sole exporter of non-coding RNAs) that plays a critical role in the microRNAs biogenesis process.[6] Evidence is emerging to show that, in both solid and liquid tumor models, CRM1 inhibition results in nuclear retention of precursor microRNAs in cancer cell nuclei.[7]

In our work published in an earlier issue of Haematologica[8] we have shown that KPT-185 treatment can result in nuclear retention of other tumor suppressor proteins. Supporting the latter findings, in a mutant p53 solid tumor model of pancreatic ductal adenocarcinoma we observed that CRM1 inhibition resulted in nuclear retention of many different major tumor suppressor proteins such as FOXO3a, IkB and an interestingly un-recognized CRM1 target: the Prostate Apoptosis Response 4 (PAR-4 or PAWR).[9] In experimental conditions, we showed that small RNA interference of PAR-4 results in substantial abrogation of CRM1 inhibitor activity. We are currently pursuing the characterization of NES sequence in PAR-4. Other studies on the mechanism of action of CRM1 inhibitor are on similar lines to those the Authors have performed on AID, although in our case the target is PAR-4. Our findings corroborate with those of Shivarov and colleagues, and confirm that there are many important players downstream of CRM1 that could be playing context-dependent roles during CRM1 inhibition-dependent apoptosis. As a caveat to this discussion, it should be noted that AID nuclear activation can have adverse effects as well. For example, Matsumoto *et al.* present a unique mechanism for hyperactivated AID-dependent p53 mutations in gastric cancer associated with *Helicobacter pylori* infections.[10] Their studies showed that *H. pylori* infection caused the aberrant expression of AID, in this context acting as a key enzyme that mediates antibody diversification, and in turn inducing p53 mutations in gastric epithelial cells.

It will certainly be interesting to see how SINE behave in the controlled systems developed by Shivarov and colleagues, i.e. where AID$^{-/-}$ BL2 cell lines are stably transfected with the AID(wt)ER or the AID(Jp8Bdel)ER transgenes. One can speculate that the cells carrying truncated AID will have enhanced activity of KPT-185. The clinical grade drug SINE KPT-330 (Selinexor®) is already in phase I trials for solid tumors and hematologic malignancies, and such experiments will provide further support for the clinical use of SINE in B-cell lymphomas.

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