Abstract #1118

Immunologically relevant effects of PT-112 on cancer cell mitochondria

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Introduction

PT-112 is a novel immunogenic small molecule [1] under clinical development for cancer therapy [2-5]. In addition to mediating cytostatic and cytotoxic effects in numerous human and mouse cancer cells, PT-112 elicits various danger signals that are linked to immunogenic cell death (ICD), such as calreticulin exposure, as well as ATP and HMGB1 secretion [3,6,7]. Accordingly, mouse cancer cells succumbing to PT-112 *in vitro* efficiently protect immunocompetent, tumor-naïve mice from challenge with living cancer cells of the same type [6,7]. Moreover, PT-112 synergizes with PD-1 or PD-L1 blockade to control mouse tumors developing in immunologically competent hosts [6,7]. In some tumor models, robust type I interferon (IFN) signaling is required for ICD [8-10]. However, the role of type I IFN signaling and mitochondria in the immunogenicity of PT-112 remains unclear.

Methods

Wild-type (WT), mitochondrial DNA (mtDNA) depleted (rho0) as well as *Casp2-/-*, *Casp3-/-* and *Bak1-/-Bax-/-* mouse hormone receptor (HR)-positive TS/A cells [11] coupled with flow cytometry, immunofluorescence (IF) microscopy and ELISA were employed to monitor PT-112-induced cell death, reactive oxygen species (ROS) generation, mitochondrial polarization, cytosolic double-stranded DNA (dsDNA) accumulation and type I IFN levels, and to investigate underlying regulatory mechanisms. In all experiments, cells were exposed to PT-112 for 24hr, unless noted otherwise. *p<0.05, ** p<0.01, ***p<0.001, ****p<0.0001 by two-tailed t-test (Fig. 3 and 7) or one-way ANOVA along with Bonferroni's multiple comparisons test (Fig. 4).

References

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Results

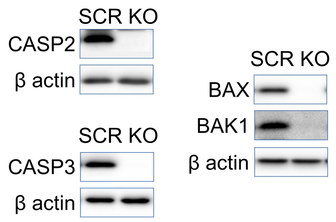


Figure 1. Generation of knockout (KO) clones in TS/A cells. Protein levels of CASP2, CASP3, BAX and BAK1 in control (Scr) and KO clones, as determined by immunoblotting and used in Fig. 2-4 and 7.

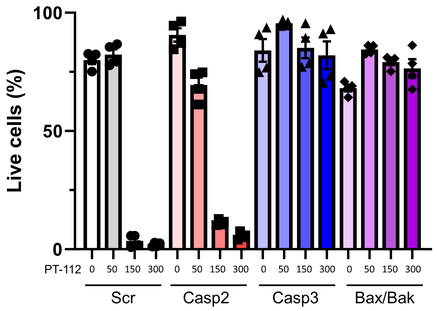


Figure 2. In TS/A cells, CASP3 and BAX/BAK1 are required for PT-112-induced cell death. Cytotoxicity of PT-112 (μg/mL) treatment. After 48hr of PT-112 treatment, the viability was measured by flow cytometry for PI uptake.

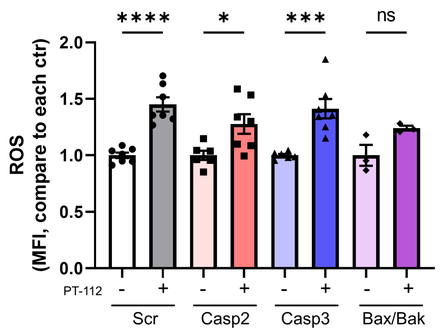


Figure 3. PT-112 mediates ROS production in part via BAX/BAK1. ROS production after PT-112 (150 μg/mL) treatment was measured by flow cytometry with a ROS-sensitive probe.

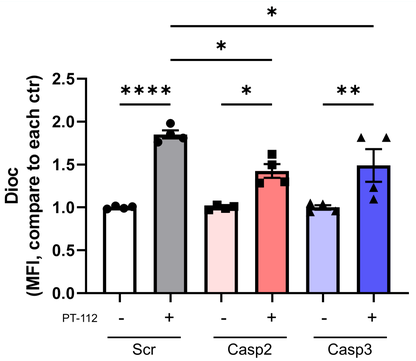
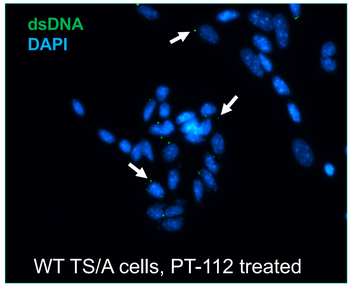
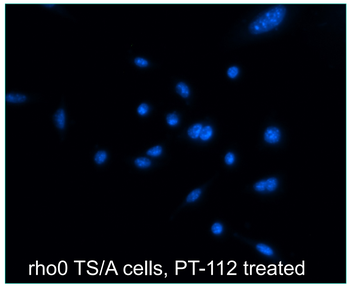


Figure 4. Mitochondrial hyperpolarization caused by PT-112 involves CASP2 and CASP3. Mitochondrial hyperpolarization after PT-112 (150 μg/mL) treatment was measured by flow cytometry upon staining with DiOC6(3) and DAPI.





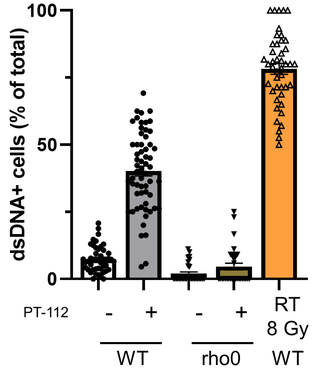


Figure 5. PT-112 induces release of mtDNA into the cytosol. Cytosolic double-stranded DNA (dsDNA) accumulation in PT-112 treated or 8Gy irradiated WT and rho0 (mtDNA depleted) TS/A cells, as assessed by IF microscopy (left, cytosolic dsDNA indicated by arrows) and absorbance-based quantification (right).

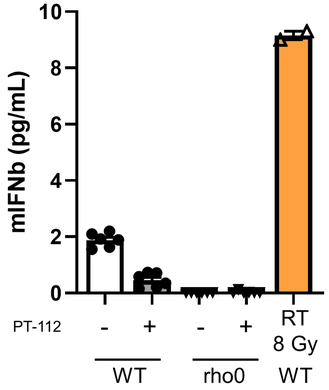


Figure 6. PT-112 does not provoke IFNB1 release in WT TS/A cells. Mouse IFNB1 levels in the supernatant of PT-112 treated or 8 Gy irradiated WT and rho0 TS/A cells.

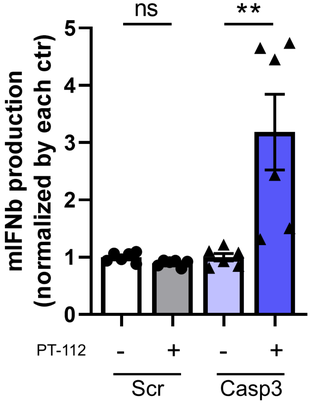


Figure 7. PT-112 provokes IFNB1 release in the absence of CASP3. Normalized mouse IFNB1 levels in the supernatant of PT-112 treated Scr1 and Casp3-/- TS/A cells.

Conclusions

- PT-112 causes pronounced mitochondrial dysfunction in cancer cells coupled with the accumulation of mtDNA in the cytosol.
- PT-112-driven ROS production is mediated in part by a BAX/BAK1-dependent pathway.
- PT-112-driven mitochondrial hyperpolarization is reduced, but not abolished by the deletion of *Casp2* or *Casp3*.
- The presence of activated BAX/BAK1 and CASP3, but not CASP2, renders TS/A cells susceptible to PT-112-induced cell death.
- CASP3 activation appears to limit IFNB1 secretion by TS/A cells responding to PT-112, suggesting that IFNB1 release may not be required in PT-112-induced ICD in this model system, as observed in our prior study [6].
- The release of IFNB1 in the absence of cell death after 24hr of PT-112 treatment in *Casp3-/-* TS/A cells suggests that PT-112 could drive immunogenic signaling before cancer cell death occurs.
- Additional studies with established tumor models are needed to clarify the impact of caspase activation on the immunogenicity of PT-112 in clinically relevant settings.