Background PT-112 is a novel platinum-pyrophosphate conjugate under clinical development for cancer therapy. Besides 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. 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. Moreover, PT-112 synergizes with PD-1 or PD-L1 blockade to control mouse tumors developing in immunologically competent hosts. In some tumor models, robust type I interferon (IFN) signaling is required for ICD. However, the role of type I IFN signaling in the immunogenicity of PT-112 remains unclear.

Methods We used wild-type, mitochondrial DNA (mtDNA) depleted (rho0), as well as Casp2−/− and Casp3−/− mouse TS/A cells. ELISA, flow cytometry and immunofluorescence microscopy were employed to monitor type I IFN levels, reactive oxygen species (ROS) generation, mitochondrial polarization, cell death, and cytosolic dsDNA accumulation driven by PT-112 and underlying regulatory mechanisms.

Results In cultured TS/A cells, PT-112 induced mitochondrial dysfunction, as demonstrated by ROS generation and mitochondrial hyperpolarization, as well as cytosolic accumulation of dsDNA that was abrogated in rho0 TS/A cells. Type I IFN secretion by wild-type TS/A responding to PT-112 was not observed. Both Casp2 and Casp3 deletion provided TS/A cells with some protection from PT-112-driven ROS generation and mitochondrial hyperpolarization, but only Casp3 deletion afforded robust protection against the acute cytotoxicity of PT-112. Importantly, both Casp2 and Casp3 deletion augmented cytosolic dsDNA accumulation driven by PT-112.

Conclusions PT-112 causes pronounced mitochondrial dysfunction in cancer cells coupled with cytosolic mtDNA accumulation, which generally leads to type I IFN secretion. PT-112-driven caspase activation, however, prevents mtDNA-driven type I IFN secretion, likely reflecting CGAS cleavage by active CASP3 and/or the rapid CASP3-dependent transition of dying cancer cells into metabolically inert corpses. In line with this possibility, both Casp2−/− and Casp3−/− TS/A cells exhibited increased cytosolic mtDNA accumulation upon PT-112 treatment. As PT-112 is a potent inducer of ICD in vaccination assays, CASP3 activation elicited during the cytotoxic response to PT-112 may not influence the ability of PT-112 to drive prophylactic anticancer immunity in tumor-naïve hosts. Additional studies with established tumor models are needed to clarify the impact of caspases on the immunogenicity of PT-112 in clinically relevant settings.

REFERENCES


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