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Targeting apoptosis inhibition to activate anti-tumor immune responses

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Summary

A recent study from Rodriguez-Ruiz et al. suggests inhibition of apoptotic caspases augments radiation-induced anti-tumor immune responses independent of type I IFN responses. Their findings also highlight caspase-independent cytotoxicity in radiation therapy-induced anti-tumor immunity and propose *SLC7A2* as a new prognostic marker for breast cancer.

Cell death is a pivotal physiological phenomenon which plays essential roles in cellular homeostasis and development [1]. Apoptosis is one such cell death pathway which allows a damaged or stressed cell to deconstruct in a regulated manner without causing inflammation. In addition to its role in physiological homeostasis, apoptosis has garnered much attention in the cancer field, with hopes of eliminating malignant cells through its activation. Apoptosis is mediated by aspartate-specific proteases called caspases [2]. Apoptotic caspases stand out as key players that decide the outcome of chemotherapy (CT) and radiation therapy (RT) [3]. However, malignant cells have adapted to employ cell-intrinsic mechanisms to counteract apoptosis and resist therapeutic cytotoxicity.

Abscopal effects have long been considered an effective way to induce systemic anti-tumor responses in RT [4]. The radiation-induced abscopal response is defined as the regression of non-irradiated tumors or metastatic lesions that are a distance away from the primary site of irradiation [4]. During RT-induced cytotoxicity, mitochondrial outer membrane permeabilization (MOMP) facilitates activation of apoptotic caspases and causes leakage of mitochondrial dsDNA into the cytosol [1, 5]. The nucleic acid sensor cyclic GMP-AMP synthase (cGAS) recognizes dsDNA in the cytosol and subsequently activates type I IFN responses to modulate immune responses [1, 5]. However, apoptotic caspases counteract cGAS activation and type I IFN responses by dismantling cells containing dsDNA. Understanding how RT-induced cytotoxicity affects anti-tumor immune responses and what processes mediate this response are still areas of ongoing research.

In a recent study appearing in *OncoImmunology*, Rodriguez-Ruiz et al. studied whether apoptosis inhibition enhances radiation-induced anti-tumor activity via type I IFNs in a breast cancer model [6]. Rodriguez-Ruiz et al. found that irradiated mouse mammary

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Disclosures

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carcinoma TSA cells undergo cell death and display apoptotic features. However, the pan-caspase inhibitor z-VAD (Z-Val-Ala-Asp fluoromethyl ketone) failed to protect TSA cells from radiation-induced cytotoxicity. To corroborate these findings, Rodriguez-Ruiz et al. genetically generated TSA cells lacking caspase-3 (CASP3), a key executioner caspase in apoptotic cell death, and found that these CASP3 knock-out (CASP3-KO) cells still show cytotoxicity in response to irradiation [6]. Interestingly, the authors found that CASP3-KO TSA clones were significantly eliminated by RT *in vivo*. These findings are intriguing as they demonstrate that apoptotic caspases are immunosuppressive during RT and CASP3 inhibition confers better therapeutic outcomes. In addition, Rodriguez-Ruiz et al. observed that CASP3-KO TSA cells accumulate cytosolic dsDNA because of a delay in the disassembly of apoptotic cells [6]. This cytosolic dsDNA promoted expression of type I IFNs, indicating that inhibiting apoptotic caspases can be immunostimulatory and boost anti-tumor functions.

To further understand the impact of CASP3 and hence apoptosis on abscopal responses during breast cancer, the authors created synchronous bilateral tumors in immunocompetent mice and subjected them to radiation-induced abscopal effects. They found that absence of CASP3 in the xenografts enhanced abscopal responses to eliminate secondary lesions when RT was combined with immune checkpoint blockade [6]. These findings establish the potential for using apoptosis inhibition to restore anti-tumor immune responses. Rodriguez-Ruiz et al. anticipated that these enhanced abscopal responses, in the absence of CASP3, are perhaps a result of elevated type I IFN responses.

To unravel the clinical relevance and translational potential of these findings, Rodriguez-Ruiz et al. analyzed transcriptional profiles from patients with breast cancer in the METABRIC public database to draw correlations between apoptosis and type I IFNs and survival in these patients. In line with their *in vitro* and *in vivo* observations, patients with lower expression of CASP3 and apoptotic peptidase-activating factor (APAF1) along with higher expression of BCL2 family anti-apoptotic proteins (BCLs) have a significant survival advantage. Furthermore, univariate Cox regression analyses indicated a high prognostic significance for CASP3 and BCLs in these patients. However, analysis showed that signatures of type I IFN responses are poorly associated with survival advantage. This suggests a disconnect between apoptosis and type I IFN response and supports the idea that the impact of apoptosis on survival for patients with breast cancer is independent of type I IFN functions.

These findings from Rodriguez-Ruiz et al. suggest that irradiated breast cancer cells still undergo cell death when CASP3 is inhibited and elicit robust abscopal responses. These observations indicate that other cell death pathways which are independent of caspases might facilitate abscopal responses in breast cancer. The radiation-induced caspase-independent cell death might be mediated by mitochondrial damage and the generation of reactive oxygen species (ROS) [1, 7]. Oxeiptosis is a unique cell death pathway induced by excessive ROS that is independent of caspases and inflammatory cell death activators [8]. Inhibition of oxeiptosis causes severe inflammation and tissue damage *in vivo* [8]. It is likely that irradiation of breast cancer cells that lack CASP3 might be activating oxeiptosis to engage anti-tumor immune responses. This is further supported by their observation that

cytosolic dsDNA induces type I IFNs, indicating mitochondrial damage and, hence, generation of ROS.

These observations also suggest that type I IFN-independent processes dominate radiation-induced abscopal responses to eliminate malignant cells. Type I IFNs are activators of inflammatory cell death pathways which release potent pro-inflammatory cytokines like IL-1 β to confer a pro-tumorigenic microenvironment [9]. Recent studies identified that ionizing radiation directly activates pyroptosis, an inflammatory form of cell death. CASP3 can also activate gasdermin E (GSDME or DFNA5)-induced pyroptosis in cancer cells [10]. Therefore, it is possible that CASP3 inhibition in breast cancer cells might inhibit specific inflammatory cell death signaling and enable caspase-independent cell death (oxeiptosis) to facilitate anti-tumor immune responses (Figure 1). This warrants further exploration.

Rodriguez-Ruiz et al. also analyzed gene expression profiles in patients with good versus bad prognosis. This analysis led them to identify *SLC7A2* (solute carrier family 7 member 2), a gene whose expression correlates with a robust survival advantage in patients with breast cancer. Based on regression analysis they further established *SLC7A2* as a novel independent prognostic biomarker for breast cancer [6]. Although a role for *SLC7A2* in controlling immune activation has been reported, further attention is required to understand its precise role in tumorigenesis and anti-tumor immunity.

Overall, this study identifies the potential for targeting apoptosis to improve the clinical efficiency of radiation therapy and eludes to the importance of studying caspase-independent cell death and type I IFN-independent mechanisms to understand the activation of anti-tumor immune responses.

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Figure 1: Apoptotic caspase inhibition in mammary carcinoma cells facilitates activation of anti-tumor immune responses and elimination of malignancy.

Radiation-induced stress in mammary carcinoma TSA cells promotes mitochondrial membrane permeabilization (MOMP) and also activation of apoptotic caspases. Radiation-induced caspase-dependent apoptosis restricts activation of anti-tumor immune responses (abscopal responses) and inhibits the release of mitochondrial DNA into the cytosol. Absence of caspase-3 (CASP3) expression in irradiated TSA cells leads to the activation of caspase-independent cell death, which robustly activates anti-tumor immune responses. Lack of CASP3 also facilitates the accumulation of cytosolic DNA that is released due to MOMP and the activation of cGAS and type I IFN responses. MOMP and generation of reactive oxygen species (ROS) might participate in caspase-independent cell death.