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The Induction of Macrophage Endoplasmic Reticulum Stress by Irradiated-Tumor Derived Extracellular Vesicles Supports the Adoption of a Pro-Tumor Phenotype

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The Induction of Dendritic Cell Endoplasmic Reticulum Stress by Irradiated-Tumor Derived Extracellular Vesicles Supports the Adoption of a Pro-Tumor Phenotype

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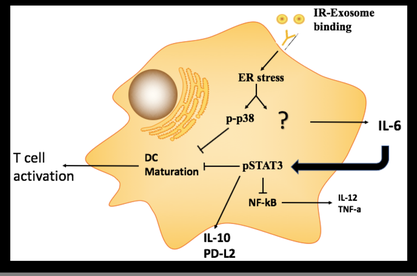
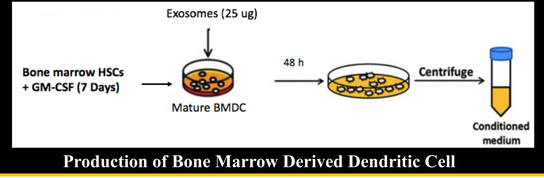
Background

•Radiotherapy is a cancer treatment that uses high doses of radiation to kill cancer cells by damaging their DNA.
•Exosomes are small extracellular vesicles produced in great numbers by cancer cells playing an important role in intercellular communication with DCs[6]. When irradiated, they produce high levels of ER stress.
•ER stress induces a pro-tumorigenic phenotypes in myeloid cells and has been demonstrated to lead to the suppression of anti-tumor immunity.
•Dendritic Cells (DCs) are known as professional antigen presenting cells necessary for anti-tumor immunity. ER stress can inhibit such immunity by causing the DCs to induce a tolerogenic phenotype.
•Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that initiates a signaling pathway that remains activated in tumors and inhibits the maturation of DCs.
•Interleukin-6 (IL-6) and Interleukin-10 (IL-10) cytokines induce a protumor phenotype in DCs.
•**Question**: Will inhibiting ER stress in cancer cells inhibit the induction of a tolerogenic DC phenotype through irradiated (IR) exosomes?

IR- exosomes induce Stat3 activity in a ER-Stress/p38 Dependent Manner



Methods



Production of Bone Marrow Derived Dendritic Cell

Abstract

Recent studies have shown that long term exposure of tumor cells to sub-lethal levels of endoplasmic reticulum (ER) stress leads to the suppression of anti-tumor immunity through the manipulation of myeloid cells in the tumor microenvironment.[1] While this effect seems to be dependent upon the ability of cancer cells to "transfer" the state of ER stress to myeloid cells, i.e. to initiate ER stress signaling in myeloid cells independent of the original stimulus, exactly how stressed cancer cells accomplish this is still not well understood[1]. Our focus is on exosomes which are extracellular vesicles and how they play a significant role in this mechanism. In recent studies, we demonstrated how extracellular vesicles secreted by irradiated melanoma cancer cells (IR-EVs) induce ER stress in Bone Marrow Dendritic Cells (BMDCs). In addition, BMDCs treated with IR-EVs demonstrated enhanced STAT3 and p38 signaling, two related pathways that have been demonstrated to induce tolerogenic DC phenotypes, in an ER stress dependent manner[2]. We've also found that IR- EVs stimulate the production of IL-10, a major negative regulator of antitumor immunity, from BMDCs and that this expression can be eliminated by STAT3 inhibition[2]. However, using a T-Cell Receptor/ tumor- associated antigen (TCR/TAA) system to model the interaction between BMDCs and cytotoxic T cells from a tumor rejection antigen (Pmel/gp100), we have observed that pharmaceutical ER stress or STAT3 inhibition dramatically inhibits T cell proliferation and IFN-gamma expression in response to antigen pulsed BMDCs. This suggests that ER stress and STAT3 signaling are both necessary for the presentation of tumor antigens to cytotoxic T cells, indicating that inhibition of these pathways would not be a desirable approach to enhance antitumor immunity in vivo.

Results

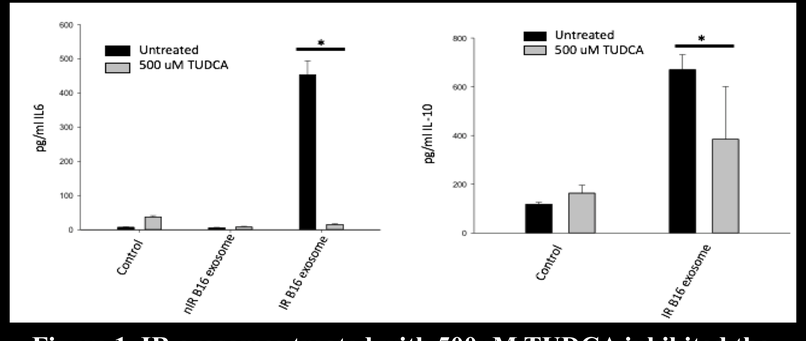


Figure 1. IR exosomes treated with 500uM TUDCA inhibited the production of IL-6 and IL-10 expression.

ER Stress caused by IR exosomes initiates a tolerogenic DC phenotype dependent upon STAT3 and P38 activation

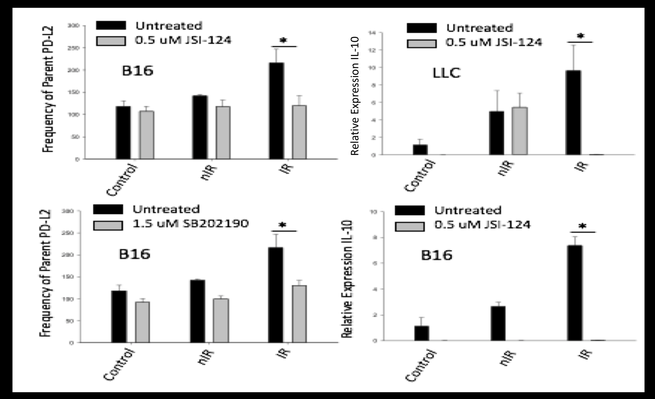


Figure 2. IR exosomes treated with a STAT3 Inhibitor (JSI-124) and P38 Inhibitor (SB202190) inhibit the frequency of PD-L2 and IL-10 expression compared to untreated IR exosomes in B16 & LLC cells.

Conclusions

•IR exosomes stimulate STAT3 & P38 expression, initiating a pro-tumor tolerogenic phenotype in DCs.
•The inhibition of ER stress inhibits STAT3 and p38 expression as well as IL-6 & IL-10 cytokines which then inhibits the production of a pro-tumor tolerogenic phenotype in DCs.
•IR exosomes stimulate STAT3 and p38 through an ER- stress dependent manner.

Future Directions

We plan to find a way to inhibit the production or activity of IR-EVs directly to inhibit their effects on DCs in the body while leaving STAT3 signaling in proliferating T-cells unaltered.

Acknowledgements

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References

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