**Ablation of ERO1A induces lethal endoplasmic reticulum stress responses and immunogenic cell death to activate anti-tumor immunity**

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**Commentator:** Dr. Chi-Wu Chiang

**Date/Time:** 2024/11/21, 16:10-17:00

**Location:** Room 601, Med College Building

**Background:** Current cancer immunotherapy techniques, among them, immune checkpoint inhibitors (ICIs), have been a revolutionary breakthrough in cancer treatment. However, due to poor clinical response after these treatments, mainly in so-called “cold” tumors, which are distinguished by low CD8+ T cell penetration, co-treatment is required to improve anti-tumor outcomes. Endoplasmic reticulum (ER) stress, triggered by the unhospitable tumor microenvironment (TME) conditions in turn signals tumor cells to respond by activating unfolded protein response (UPR), which reinstates ER function, facilitating tumor growth and drug resistance instead of undergoing immunogenic cell death (ICD). During this process, the expression of endoplasmic reticular oxidoreductase-1α (ERO1A) is increased, which aids protein folding and alleviates ER stress, resulting in overall tumor cell survival. It has been understood previously that ERO1A activation drives immunosuppression, however the mechanisms by which ERO1A contributes to immune suppression are still poorly understood.

**Objective:** To investigate the roles of ERO1A in immune suppression attributed to TME remodeling and to target ERO1A as an inhibitor of ER stress and as a therapeutic biomarker to turn “cold” tumors to “hot” ones in response to immunotherapy.

**Results:** Using Ero1a knockout in various cell lines and treatment with anti-PD-1 (programmed cell death protein 1) drug, tumor growth suppression was greater and tumor-infiltrating cytotoxic lymphocytes were increased compared to cells with wild-type ERO1a, suggesting that ERO1A ablation increases anti-tumor immunity and responses to PD-1 blockade. Next, using the TCGA database, they found inverse correlations of ERO1A mRNA expression and clinical outcomes such as patient survival in various cancers and genes correlating anti-tumor immunity. Single-cell RNA sequencing (scRNA-seq) analysis revealed that an abundance of active CD8+ T cells were identified in the *Ero1a* knockout tumors which provide a more effective anti-tumor immunity, while M2 and M1 tumor-associated macrophages (TAMs) are more abundant in the wild-type and Ero1a knockout tumors, respectively, indicating the role of ERO1A in promoting phenotype transition of TAMs. This confirmed that ERO1A promoted immunosuppression by TME remodeling. Further analysis using GSEA also revealed that Ero1a knockout in tumor cells is able to upregulate lethal ER stress pathways and induce immunogenic cell death (ICD) by overexpression of damage-associated molecular pattern (DAMP)-related genes in vivo, thereby improving tumor-free survival. Further sequencing analysis also revealed that mechanistically, Ero1a knockout tumor cells exhibited upregulated UPR pathway genes with defects in activation of IRE1α pathway, results in unresolved ER stress. Finally, clinical studies demonstrated that patients with low ERO1A expression have a significantly better clinical outcome after aPD-1 immunotherapy together with a longer relapse-free survival.

**Conclusion:** ERO1A plays a significant role in alleviating ER stress in tumor cells via the IRE1α UPR pathway and in the dysfunction of T cells by TME remodeling in various types of cancer. By ablation of ERO1A, unresolved ER stress induces the ICD pathway, therefore improving patient clinical outcomes. With this, ERO1A can be targeted as a biomarker of cancer outcomes after immunotherapy.