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“Evaluation of Immunogenic Cell Death, Proliferation, and Epithelial-Mesenchymal Transition Following Decorin-Expressing Oncolytic Virotherapy in Pancreatic Ductal Adenocarcinoma Cells”

Pancreatic ductal adenocarcinoma (PDAC) is characterized by a dense, desmoplastic tumor stroma that contributes to therapeutic resistance and limits treatment efficacy. Decorin is a proteoglycan that has previously been shown to modulate the PDAC tumor microenvironment by reducing fibrosis. To enhance the therapeutic potential of oncolytic virotherapy, we engineered an attenuated vesicular stomatitis virus (VSV) expressing human decorin (VSV-DCN). Previous studies from our laboratory demonstrated that VSV-DCN selectively reduces the viability of PDAC cell lines while sparing normal pancreatic cells (H6C7) following 72 hours of infection.

We hypothesized that decorin expression modifies tumor-cell responses to oncolytic VSV, including proliferation, EMT-associated phenotype, and calreticulin expression. We infected the human pancreatic cell lines ASPC-1 and MiaPaCa-2 with mock treatment (control), VSV-GFP (viral backbone), or VSV-DCN. Immunofluorescence was performed to evaluate calreticulin as a hallmark marker of immunogenic cell death (ICD), along with Ki-67 to assess proliferation and E-cadherin and vimentin to characterize changes associated with epithelial–mesenchymal transition (EMT). Quantitative analysis of immunofluorescence images was performed using StarDist and ImageJ. StarDist was used to quantify nuclei number from DAPI counterstained images, while ImageJ was used to measure the integrated density of the marker-specific immunofluorescence images. Integrated fluorescence density was normalized to nuclei count to estimate marker expression per cell and reduce variability resulting from differences in cell density between images.

Across the three experimental conditions, VSV-GFP produced variable effects on marker expression relative to untreated controls. Notably, VSV-GFP reduced calreticulin expression by 54.1% in ASPC-1 cells at MOI 1.0 and by 39.4% in MiaPaCa-2 cells at MOI 1.0, while producing minimal change (1.6% decrease) in MiaPaCa-2 cells at MOI 0.1. To distinguish effects attributable to the VSV backbone from those associated with decorin expression, we next compared VSV-DCN directly with VSV-GFP. Relative to the viral backbone, VSV-DCN reduced Ki-67 expression by 35.3% in MiaPaCa-2 cells at MOI 1.0 and 22.2% at MOI 0.1, while increasing Ki-67 expression by 12.5% in ASPC-1 cells at MOI 1.0. VSV-DCN also increased calreticulin expression by 65.8% in ASPC-1 cells at MOI 1.0 and 55.4% in MiaPaCa-2 cells at MOI 0.1, while decreasing calreticulin expression by 48.7% in MiaPaCa-2 cells at MOI 1.0. Altogether, VSV-DCN was associated with reduced Ki-67 expression compared to control groups across the conditions tested, although the relative contribution of decorin versus the VSV backbone varied by model. No consistent changes in E-cadherin or vimentin were observed. Calreticulin expression differed between VSV-DCN and VSV-GFP under select conditions, supporting further evaluation of decorin-mediated effects on ICD-associated signaling using additional functional markers of immunogenic cell death.