

Examination of Wnt signaling as a therapeutic target for pancreatic ductal adenocarcinoma (PDAC) using a pancreatic tumor organoid library (PTOL).

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Background: Pancreatic ductal adenocarcinoma (PDAC) commonly presents at advanced stages and is refractory to most treatment modalities, making it one of the most lethal cancers. Although the low tumor cellularity and high desmoplastic response convolutes the relationship between genotype and biological phenotypes, gene mutations associated with PDACs have been identified. Wnt pathway mutations are rarely detected in PDAC, but Wnt signaling is activated by pancreatic duct ligation injury and plays a critical role in the proliferation and chemotherapeutic resistance in other cancers. Patient derived pancreatic tumor organoid libraries (PTOL) allow for more accurate of the biological phenotypes that might lead to therapies that further improve survival. This study aims to subclassify PDAC organoids based on Wnt dependency and determine if combinatory treatment with Wnt inhibitors and chemotherapy would serve as a feasible treatment.

Material and Methods: Minimal media conditions required to maintain growth of nine PDAC organoids grown in Human Pancreatic Stem Cell medium was assessed with depletions of various niche factors. For confirmation of Wnt inhibition, organoids grown in minimal media were treated with Wnt inhibitors (ETC-159, ICG001, C59). Select organoids demonstrating Wnt dependency were treated with the Wnt inhibitor ETC-159 as a single agent and in combination with gemcitabine or paclitaxel *in vitro*. Growth was assessed with CellTiter Glo 3D and ANOVA was used for statistical analysis. Organoid lines demonstrating response to combinatory treatment *in vitro* were assessed *in vivo* as a matched patient-derived xenograft. Wnt (in)dependent gene signatures were identified for each organoid and RT-PCR was used to determine fold change gene expression of key Wnt genes in tumors following *in vivo* treatment.

Results: Minimal media conditions, growth factor dependency, and Wnt dependency determined via Wnt inhibition were determined as described above for seven patient derived organoids (PDOs): Panc129, Panc193, Panc268, Panc269, Panc272, Panc308, Panc320. Panc269 demonstrated a trend of reduced organoid growth when treated with ETC-159 in combination with paclitaxel or gemcitabine as compared with chemotherapy or ETC-159 alone. Panc320 demonstrated a more pronounced anti-proliferative effect in the combination of ETC-159 and paclitaxel but not with gemcitabine. Panc269 and Panc320 were implanted into nude mice and treated with ETC-159, paclitaxel, and gemcitabine as single agents and in combination. The combination of ETC-159 and paclitaxel demonstrated an anti-tumor effect greater than ETC-159 alone. Extent of combinatory treatment effect were observed to a lesser extent in the Panc320 xenograft. Wnt (in)dependent gene signatures of Panc269 and 320 were consistent with the phenotypes displayed, and gene expression of several key Wnt genes also demonstrated notable fold change following treatment *in vivo*.

Conclusions: Based on the results obtained, each pancreatic organoid demonstrated varied niche factor dependencies providing an avenue for targeted therapy, particularly with Wnt inhibition, which was supported through growth analysis following combinatory treatment of Wnt inhibitor and standard chemotherapy *in vitro*. The clinical utilization of this combinatory treatment modality in pancreatic cancer PDOs has thus far been supported in our patient-derived xenograft models treated with Wnt inhibitor plus paclitaxel or gemcitabine. Gene expression analysis suggests that there are key Wnt genes that contribute to the Wnt (in)dependent phenotypes of pancreatic tumors, providing plausible mechanistic explanation for Wnt (in)dependency and susceptibility or resistance to treatment on the genotypic level.