Background & Introduction

- Pancreatic cancer cannot be cured without surgically removing the tumor from the pancreas. Only 10-15% of patients can have an operation because of late-stage diagnosis.
- The small intestine is one of the most radiosensitive tissues in the body. Due to the already sensitive nature of the tissue and the proximity to the pancreas there is great risk during pancreatic cancer radiation treatments.
- The Taniguchi Lab previously found that stabilizing the expression of hypoxia-inducible factor 2 (HIF2) can improve survival in mouse models of radiation injury; however, the mechanisms of the effect are still unknown.

Methods

Preparation of human intestinal tissue (Fig. 2):
- Duodenal enteroids were generated from tissue obtained from patients during gastric bypass surgery through Baylor School of Medicine and MD Anderson. The intestinal stem cells were isolated and grown using the Clevers’ protocol.

Determining the role of HIF2 in the radiation response of human intestinal tissue:
- Enteroids were treated with hypoxia mimicking drug, FG-4592 (50uM), which inhibits the prolyl hydroxylation of HIF2. They were incubated, and at 24-, 48-, and 72- hours cell lysates for protein expression analysis were prepared for HIF2 immunoblotting (1:500, NB100-122). We also dissected the downstream mechanism of HIF2 pathway by using a HIF2 antagonist, PT-2399. We optimized the combination treatment of PT-2399 (1.0uM) along with FG-4592 (50uM) for gene analysis by quantitative PCR.

Results

- Treatment with the hypoxia mimic, FG-4592, or HIF2 inhibitor, PT-2399, induced no morphological differences or toxicity in the enteroids (Fig. 3). Immunoblot analysis shows that HIF2 expression is maximally stabilized after 72 hours (Fig. 2).
- Since we did not find any changes in protein levels of target gene Wnt5a so we looked at the mRNA level. qRT–PCR analysis was performed for hypoxia target genes. This demonstrated an upregulation in WNT5A and PGK1 after treatment with FG-4592 (50uM) (Fig. 5).

Discussion

- Treatment related gastrointestinal (GI) toxicity is likely the greatest barrier to improving treatment responses for unresectable pancreatic cancer. Efforts to improve patient outcomes prompts the Taniguchi lab focus on radioprotection.
- Exploring the GI organoid phenotypes for study of HIF2 pathway induced by EGLN inhibition is a step towards understanding the cellular changes taking place during radiotherapy.
- Continuing this approach should allow further characterization of human enteroids to understand the therapeutic capacity of EGLN inhibition to reduce radiation toxicity.

References

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