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Rac1: a novel diagnostic marker for precision medicine in pancreatic cancer?

Pancreatic ductal adenocarcinoma (PDAC) is among the deadliest cancers, characterized by high capacity for metastasis, immune suppression and resistance to therapy. Upon diagnosis nearly 55% of patients already suffer from distant metastases. This highlights the crucial need for improved biomarkers and therapeutics; in order to inhibit metastasis and drug resistance, while boosting the immune system against the disease.

This study, as part of a Master's thesis, investigated whether Rac1 gene influences migration, nutrient uptake, viability, therapy resistance, potential immune evasion mechanism and EMT profile in cell culture.

Macropinocytosis is a specialized form of endocytosis which cancer cells use to take up nutrients. Rac1 Overexpression in our cell types increased macropinocytosis in dextran uptake assay, resistance to Gemcitabine in MTT and migration capacity in scratch assays in comparison to the wild-type.

Inhibiting Rac1 via EHT1864 hindered the cancerous features of Rac1 overexpression markedly in wild-type cells. Rac1 Knockdown resulted in effects similar to pharmacological inhibition in BXPC3 lines, with some distinction in Colo 357 lines. Rac1 inhibition clearly enhanced the cytotoxicity of Gemcitabine in both wild cell types.

Gal-9 is a protein secreted by both immune & tumor cells; it induces apoptosis in immune cells and increases immune escape. Rac1 expression, indirectly, influenced the amounts of secreted Galectin-9 in the ELISA experiment. Colo357 lines showed a decreasing trend of soluble Gal-9 from Rac1 OE to WT to Rac1 KD; in contrast, BXPC3 Rac1OE had much less soluble Gal-9 than BXPC3 WT and BXPC3 Rac1 KD. Regarding EMT markers in FACS, a heterogenous expression of Gal-9 was observed but Cytokeratin 19 (internal epithelial marker) expression was homogenous, which was increased in Rac1 OE samples.

We also re-confirmed the presence of Rac1 and Gal-9 in our FFPE patient samples via HRP IHC.

Our findings suggest that Rac1 plays an active role in cell migration, Macropinocytosis, survival, therapy resistance and potential immune-escape in vitro. Rac1 inhibition leads to desired anti-tumor effects; suggesting Rac1 as a feasible candidate for biomarker research and therapeutic intervention in vivo, hinting to the value of precision medicine. However, its translational value is up for debate.

Key Words: PDAC, Rac1, Macropinocytosis , Gemcitabine, EHT1864, Dextran, Viability, Migration