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The Role of PAF1/PD2 in Inducing Drug Resistance In Pancreatic Cancer Cells

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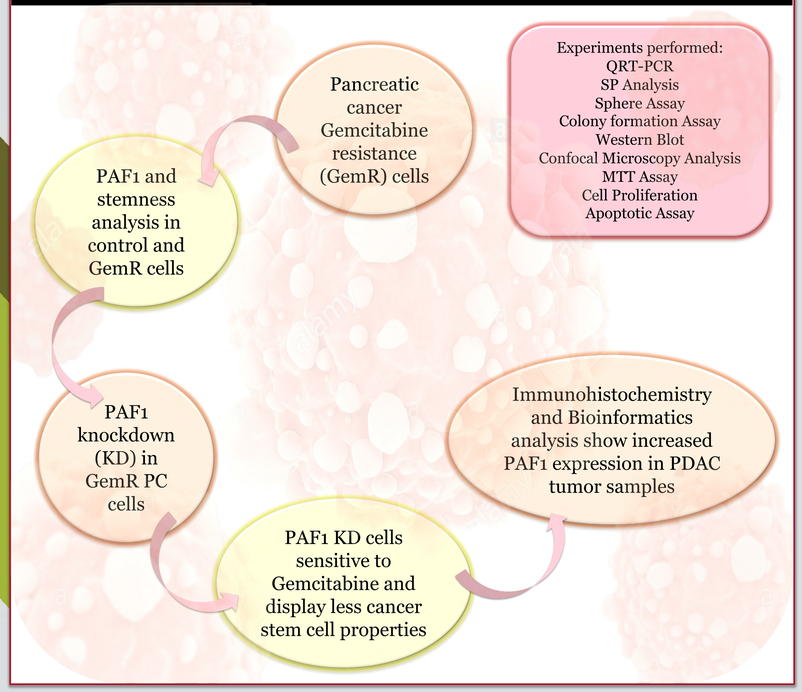
BACKGROUND

- Pancreatic cancer (PC), a highly aggressive human cancer, is the third leading cause of death due to cancer, with a five-year survival rate[1].
- Cancer stem cells (CSCs) are a small and distinct population of cancer cells that mediate tumorigenesis, metastasis and resistance to standard treatments[1].
- Specifically identifying and targeting CSC maintenance genes can improve the efficiency of treatment modalities[5].
- PAF1 (RNA Polymerase II-Associated Factor 1), also known as PD2 (Pancreatic Differentiation 2), is the core subunit of the human PAF1 complex (PAF1C). It maintains pluripotency of stem cells and is a marker of pancreatic CSCs[2 3].
- PAF1/PD2 is upregulated in poorly differentiated pancreatic cancer cells[2].
- Gemcitabine (Gem) is a novel deoxycytidine analogue developed as an anticancer therapy[4]. It is widely used as a chemotherapeutic agent and is presently the most effective agent against pancreatic cancer[5].

HYPOTHESIS

PAF1/PD2 plays a role in the maintenance of Pancreatic cancer stem cells and contributes to Gemcitabine resistance.

EXPERIMENTAL DESIGN AND METHODS



RESULTS

Gemcitabine increases expression of PAF1/PD2 and CSC Markers

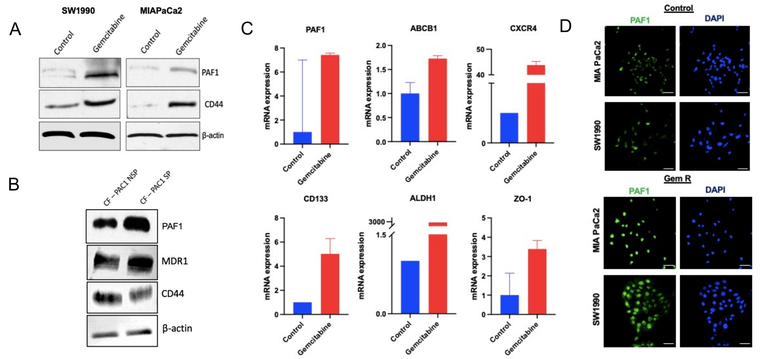


Fig.1: A) Western blot depicting the Gemcitabine treated population exhibiting a higher expression of CD44 and PAF1/PD2 in comparison to the control population. B) Western blot depicting SP cells exhibiting a higher expression of PAF1, MDR1 and CD44 in comparison to NSP cells. C) Addition of Gemcitabine caused a significant increase in the expression of CSC markers (PAF1, ABCB1, CXCR4, CD133, ALDH1, and ZO-1). D) Confocal microscopy analysis of a Gemcitabine resistant population which shows a higher expression compared to the control population in MIAPaCa2 and SW1990 PC cell lines.

Loss of PAF1/PD2 influences maintenance of PC cells and sensitizes the cells to Gemcitabine

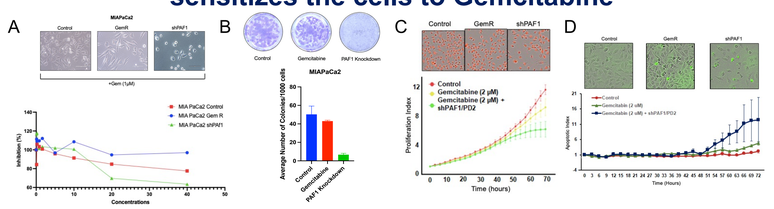


Fig.2: A) MTT Assay depicting the knockdown of PAF1/PD2 leading to a decrease in the MIAPaCa2 PC cell line as compared to the control and Gemcitabine resistant populations. B) *In vitro* colony formation tumorigenesis assay performed using 1.0 x 10^3 PC cells which were fixed and stained after 14 days in culture. C) Cell proliferation analysis displaying a decrease in cells in PAF1/PD2 knockdown compared to the control group. D) Apoptotic Assay depicting higher cell death with PAF1/PD2 knockdown.

Impact of PAF1/PD2 on Gemcitabine Resistance and CSC marker expression

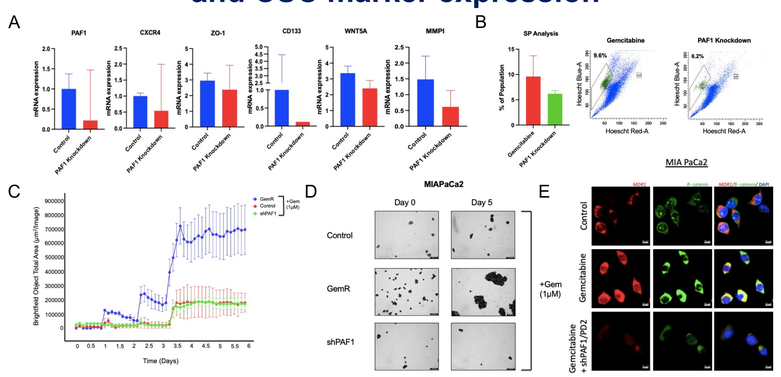


Fig.3: A)) Knockdown of PAF1/PD2 led to a decrease in the expression of CSC markers (PAF1, CXCR4, ZO-1, CD133, WNT5A, and MMPI) B) Fluorescent activated cell-sorting (FACS) analysis of PC cells stained with soluble Hoescht dye in order to determine % of population of Pancreatic CSCs (side population analysis). C) and D) Sphere Assay analysis depicts the knockdown of PAF1/PD2 significantly caused a decrease in the MIAPaCa2 PC cell line in comparison to the control and Gemcitabine resistant populations. E) Confocal microscopy analysis of a PAF1/PD2 knockdown population which shows a lower expression of MDR1 and β-catenin compared to the control population in the MIAPaCa2 PC cell line.

RESULTS

Impact of PAF1/PD2 on Human Pancreatic tumor samples

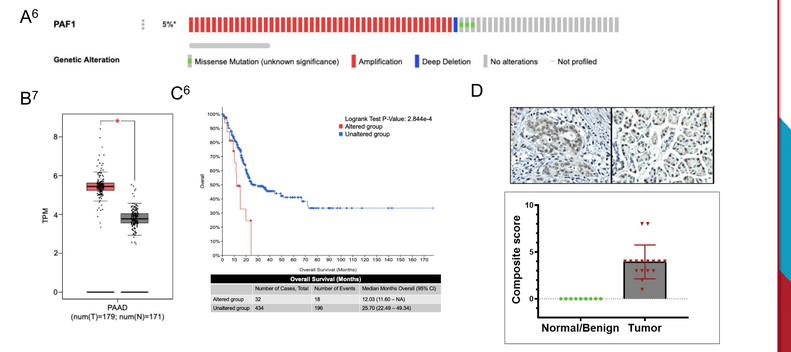


Fig. 4: A) Data obtained from cBioPortal demonstrating that 5% of PC patients out of 100 were shown to have higher levels of PAF1. B) Data obtained from Gepia demonstrating that tumor patients had higher levels of PAF1/PD2 in comparison to normal patients. C) The survival rate comparison between the altered group (samples with at least one alteration in PAF1 in PC patients) and unaltered group (samples without any alterations in PAF1 in PC patients) over a period of 178 months. D) Composite score comparison of PAF1/PD2 between normal/benign tumor vs. malignant tumor depicting higher levels of PAF1/PD2 in a malignant tumor.

CONCLUSION

- PAF1/PD2 are over expressed in Pancreatic Tumor cells, specifically in cancer stem cells (CSCs).
- Increased expression of PAF1/PD2 is associated with gemcitabine resistance in pancreatic cancer cells.
- The knockdown of PAF1/PD2 leads to a significant reduction in expression of CSC markers and pancreatic tumorigenesis.
- Human pancreatic tumor samples showed increased expression of PAF1/PD2. Additionally, altered expression of PAF1/PD2 has prognostic relevance to pancreatic cancer patient survival.

FUTURE DIRECTIONS

- Identify the specific inhibitor for PAF1/PD2 using an *In-silico* analysis
- Test the efficacy of PAF1/PD2 inhibitor in cancer stem cell and drug resistant models
- Investigate the therapeutic efficacy of PAF1/PD2 inhibitor along with gemcitabine using preclinical models

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