Transcriptomic Analysis of Exosome Cargo Derived From Pancreatic Cancer and Stellate Cells: Prospective Mediators of Pancreatic Cancer-Related Diabetes.

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Background

- Pancreatic cancer-related diabetes (PCRD) could be a harbinger of asymptomatic PC.
- The earliest lesions of pancreatic cancer, pancreatic intraepithelial neoplasms (PanINs) are surrounded by pancreatic stellate cells (PSCs) that produce the collagenous stroma of PC.
- Interaction of PSCs and PC/PanIN cells facilitates PC progression.
- Exosomes/small extracellular vesicles (40-160nm) are gaining attention as important mediators of intercellular communication in all stages of cancer.

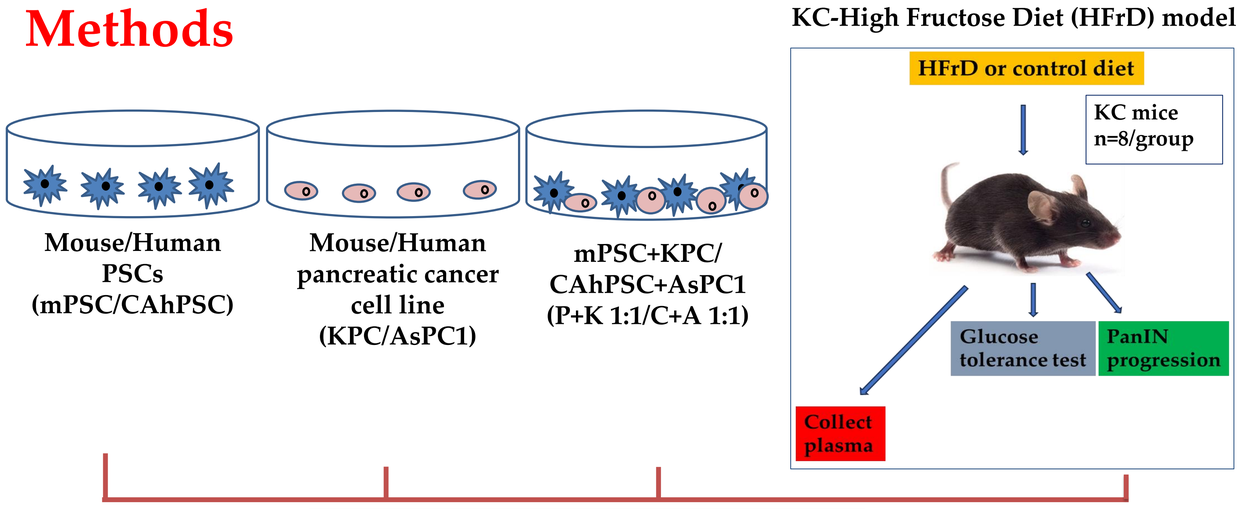
Hypothesis

PSC-cancer cell interactions lead to secretion of exosomes containing unique RNAs that mediate PCRD.

Aim

To identify and characterise the RNA cargo within exosomes derived from i) PSCs and PC cells cultured alone and together and ii) plasma from a mouse model of PCRD.

Methods



Cells and plasma samples: n=3-4/group

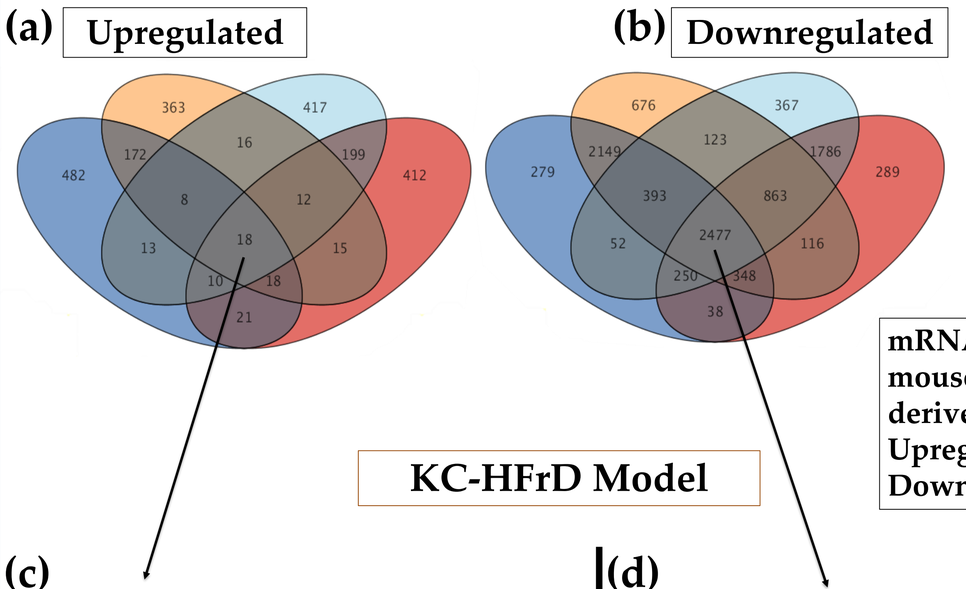
Results

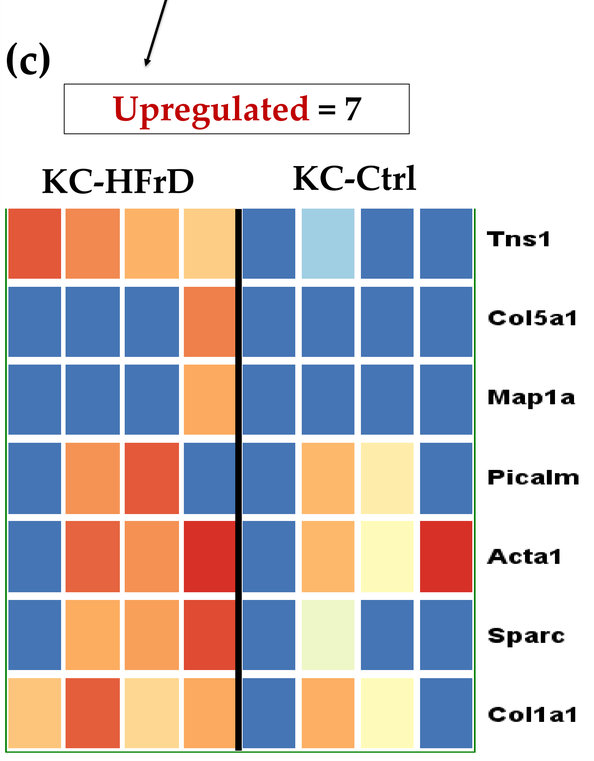
1) Exosome Characterisation

(a) Representative TEM image of PSC+KPC (P+K) coculture exosomes demonstrating typical cup shaped morphology and size. (b) Exosomes (Ex) were positive for exosome specific markers (ALIX, TSG101, HSP70) and negative for the non-exosomal (endoplasmic reticulum) marker, Calnexin which was present only in cell lysates (CL). (c) P+K exosomes gene type plot showing expression of both protein coding and non-coding RNAs.

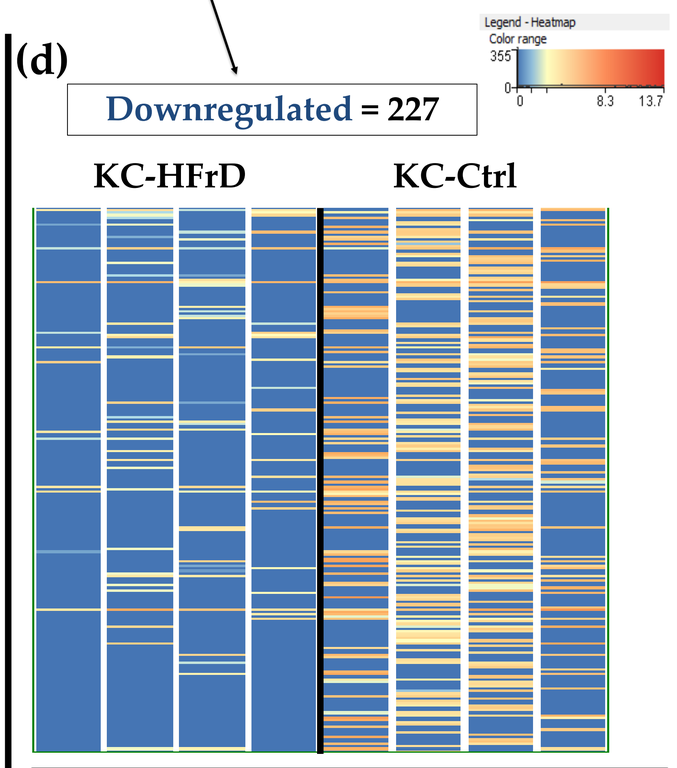
Results (continued)

2) RNA-Seq: Differentially Expressed mRNAs in Mouse and Human Cell-Derived Exosomes





Of the upregulated mRNAs, PICALM is associated with impaired glucose tolerance.

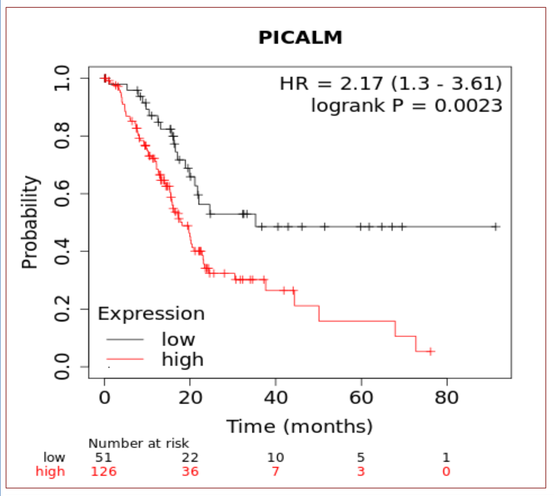


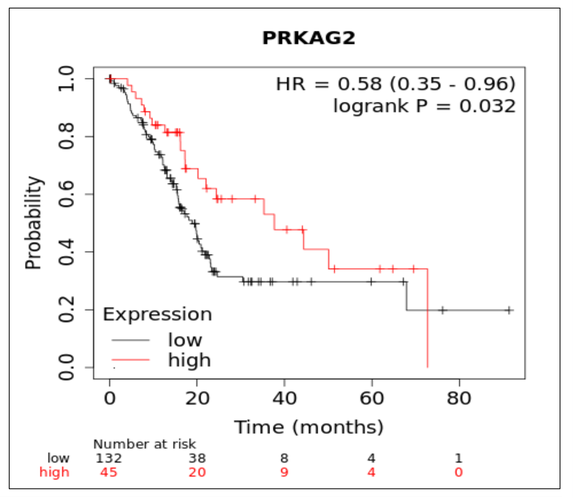
Of the downregulated mRNAs, PRKAG2 is associated with insulin signalling and insulin resistance.

PICALM (phosphatidylinositol binding clathrin assembly protein) was upregulated in PSC+KPC and CAhPSC+AsPC1 cocultured exosomes (Fig 2a), and in plasma of glucose intolerant (KC HFrD) mice (Fig 2c).

PRKAG2 (Protein kinase AMP-activated non-catalytic subunit gamma 2) was downregulated in PSC+KPC and CAhPSC+AsPC1 cocultured exosomes (Fig 2b), and in plasma of KC HFrD mice (Fig 2d).

3) Candidate mRNA Expression and Patient Survival (TCGA)





High expression of PICALM correlated with poor survival of pancreatic cancer patients.

Low expression of PRKAG2 correlated with poor survival of pancreatic cancer patients.
TCGA: The Cancer Genome Atlas.

Conclusions and Implication

This is the first study to:

- Characterise the exosomal RNA cargo of cocultures of PSCs and cancer cells
- Demonstrate that exosomes derived from PSC-PC coculture and from glucose intolerant KC mice, are enriched in specific mRNAs known to modulate pathways that play a role in diabetes and/or PC.

Functional validation of PICALM and PRKAG2 may identify these factors as novel biomarkers and/or therapeutic targets for pancreatic cancer.