Investigation of Novel Biomarkers for Pancreatic Cancer

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Introduction

Pancreatic cancer (PaCa) is among the deadliest cancers with only 3% of diagnosed patients given a 5-year survival rate. It is diagnosed at a late stage mostly due to the lack of appropriate biomarkers for early diagnosis. CA19.9 is a currently used biomarker but does not exhibit the desirable level of specificity and sensitivity. In order to identify novel potential biomarkers we used a lipidomics-based approach and focused our attention to lysophospholipids and enzymes involved in their metabolism as suitable novel PaCa biomarker candidates. We are currently investigating pancreatic cancer cell lines compared to normal pancreatic lines by lipid isolation from PaCa cell lines supernatant, and transgenic mouse models generating the full PanIN lineage, and blood and urine samples from PaCa patients and healthy controls. Our group has previously shown that Ras-transformed cells exhibit elevated lysophospholipids release able to stimulate cancer autocrine cell proliferation. Moreover, our group has suggested an autocrine loop in which ABCC transporters efflux lysophosphatidylinositol (LPI) which in turn promotes cell proliferation via GPR55 activation. Our goal is to determine whether there is a lipid signature unique for PaCa, and make a comparison with other cancer types, such as ovarian and prostate cancer. Determining an effective biomarker can replace costly and prohibitive for routine screening, technologies.

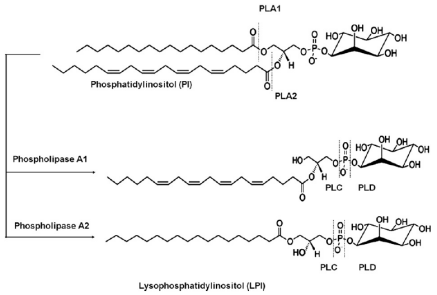

***Figure 1*: LPI synthesis from phosphatidylinositol (PI)**

Methods

- Incorporation of [^{3}H]*myo*-Inositol and detection of radiolabelled lipids: Cells were incubated in FBS free media containing [^{3}H]*myo*-Inositol for 48 hours, washed with PBS and incubated in Hanks' Balanced Salt Solution (HBSS). Lipids present in the supernatants were extracted through incubation with a mixture of CH3OH/CHCl3/HCl 12N. Radioactivity released was measured by liquid scintillation.
- Transient siRNA knockdown targeting ABCC3 and cPLA2: After transfection, cells were metabolically radiolabelled and incubated with +/- EGF for 60 minutes. Lipids were extracted from cell supernatants by phase separation and radioactivity assessed by scintillation counting.
- LPI stimulation of cell growth: Cells were incubated with the indicated concentration of LPI and cell viability was determined 72 hours later by manual counting

Conclusions

- In contrary to prostate cancer cells, PDAC cells use ABCC3 transporter to pump LPI out of the cells and not ABCC1, and this is mainly stimulated by EGF
- Exogenous LPI acts as a mitogenic factor in pancreatic ductal adenocarcinoma cells
- Downregulation of cPLA2 and/or ABCC3 impaired cancer cells growth - indication of importance of endogenous LPI
- LPI may regulate PDAC cell growth by activating the MAPK/ERK signalling pathway (data not shown)

Future perspectives

- Determination of the precise mechanism by which EGF stimulates LPI release and whether the fatty acid remodelling pathway is involved in PDAC cells
- Identification of the specific LPI species released by pancreatic cancer cells and the pathway responsible for LPI synthesis and release
- Investigation of LPI production in transgenic mouse models of PDAC
- Investigation of LPI production in plasma and urine samples from normal and pancreatic cancer patients
- **Establishment of lysophospholipids and enzymes involved in their metabolism as suitable novel PaCa biomarker candidates**

Results

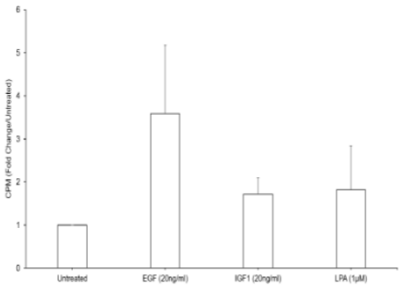

***Figure 2:* [^{3}H]myo-Inositol-containing lipids released by HPAFII cells upon stimulation with different growth factors.**

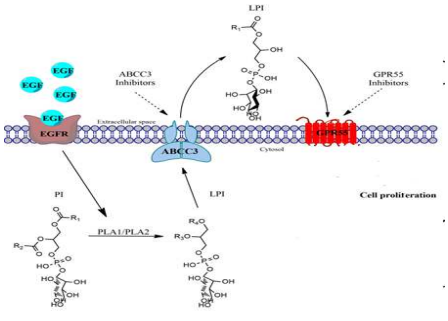

***Figure 3:* Scheme of the signalling loop involved in HPAFII cell line**

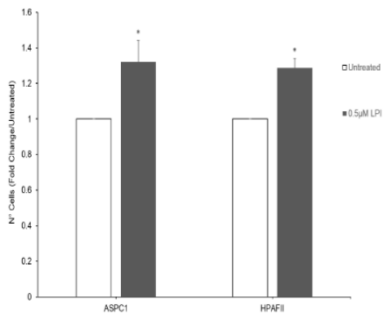

***Figure 4:* Effect of LPI on ASPC1 and HPAFII cell growth**

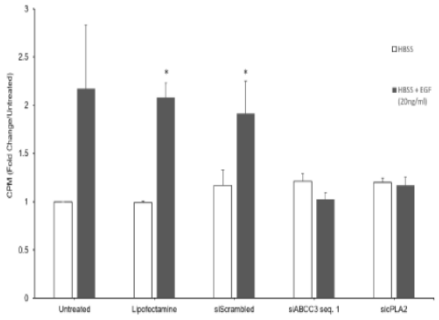

***Figure 5:* ABCC3 and cPLA2 downregulation impairs lipids release upon EGF stimulation in HPAFII**

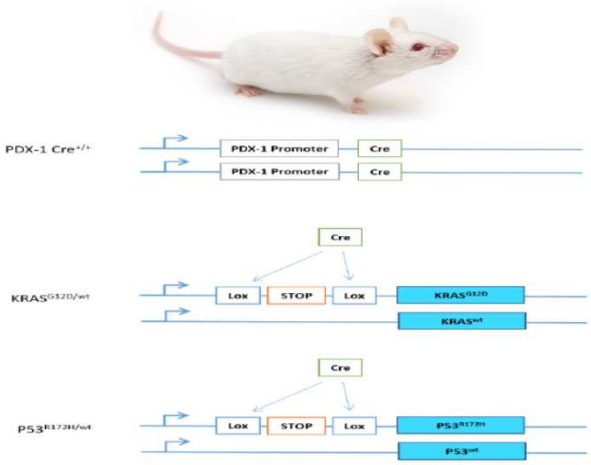

***Figure 6:* Transgenic mouse models - Mutant KRAS/P53 expressions localised to the pancreas via LSL - Cre recombinase system**

References

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