

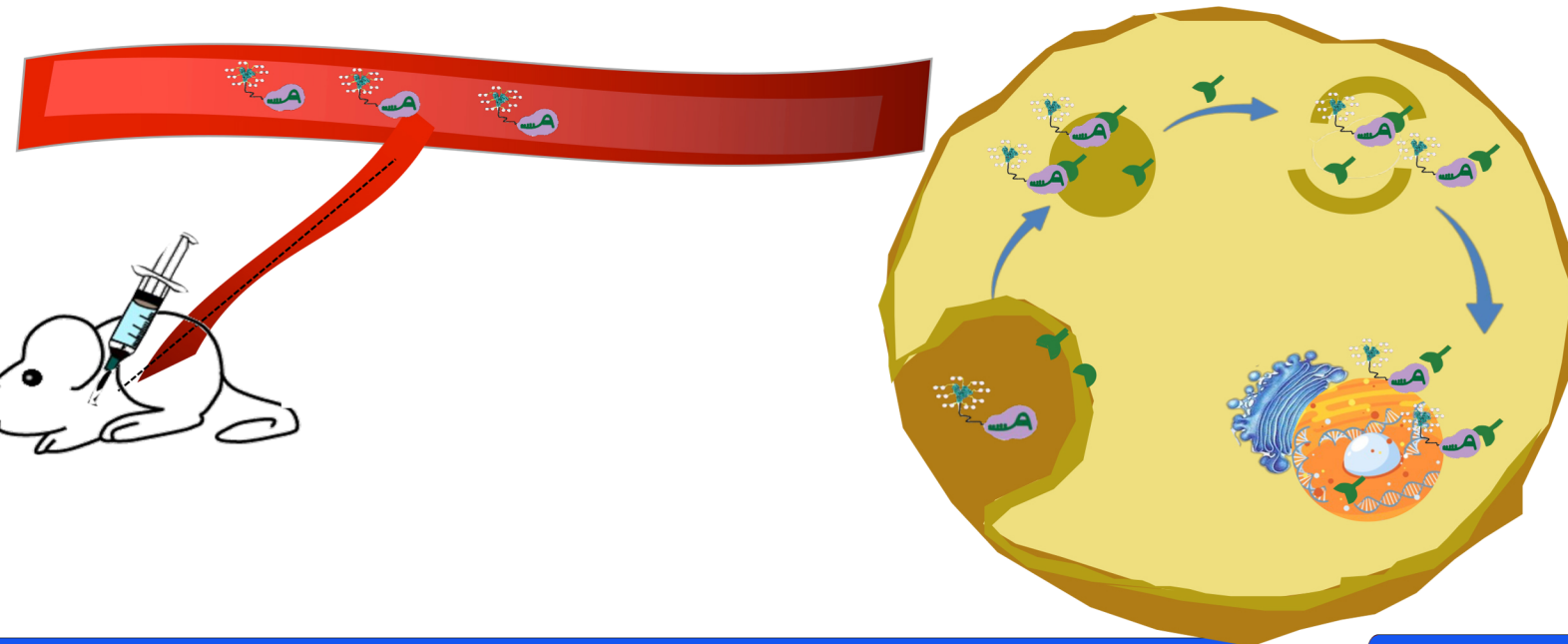
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Background: Receptor tyrosine kinases (RTKs) from different families translocate from the cell surface to the nucleus; PDGFR- β enters the nucleus through the nuclear pore complex depending on the recognition and transport by β -importin. pPB (a cyclic peptide) can specifically recognize PDGFR- β and carry drugs into fibroblasts.

Aim: To investigate whether pPB can co-deliver bioactive molecules into the nucleus by harnessing the PDGFR- β nuclear translocation pathway.

Introduction

Liver fibrosis: Pathogenesis, target cells, and pPB-HSA therapy

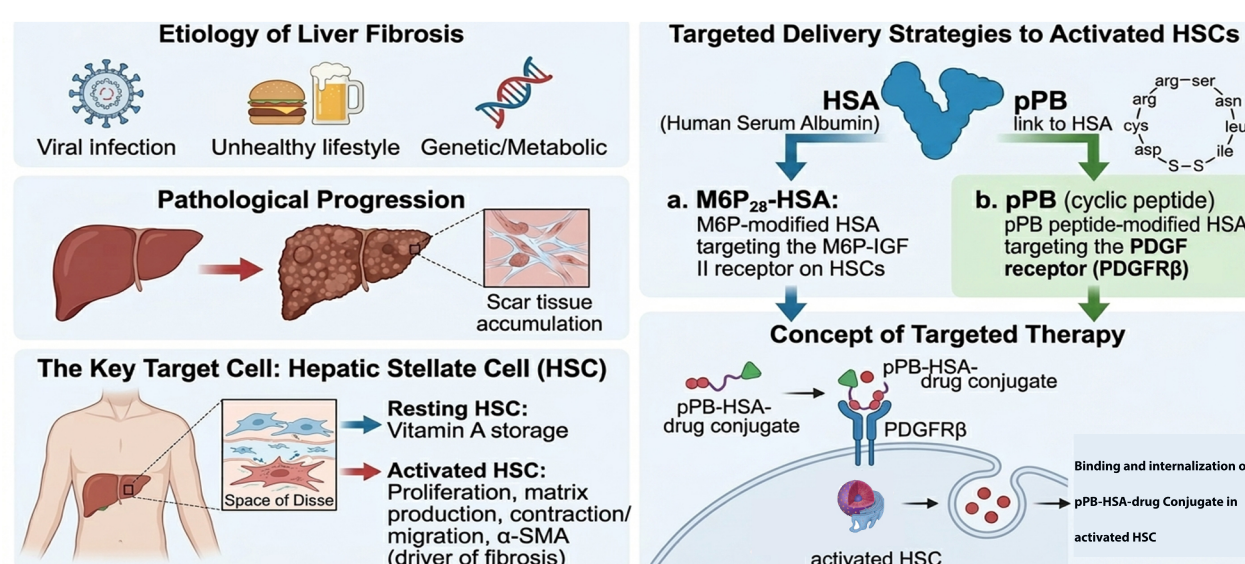


Fig1. Schematic overview of the liver fibrosis pathogenesis and the pPB-HSA targeted therapy strategy.

Methods

Nuclear isolation using nitrogen cavitation

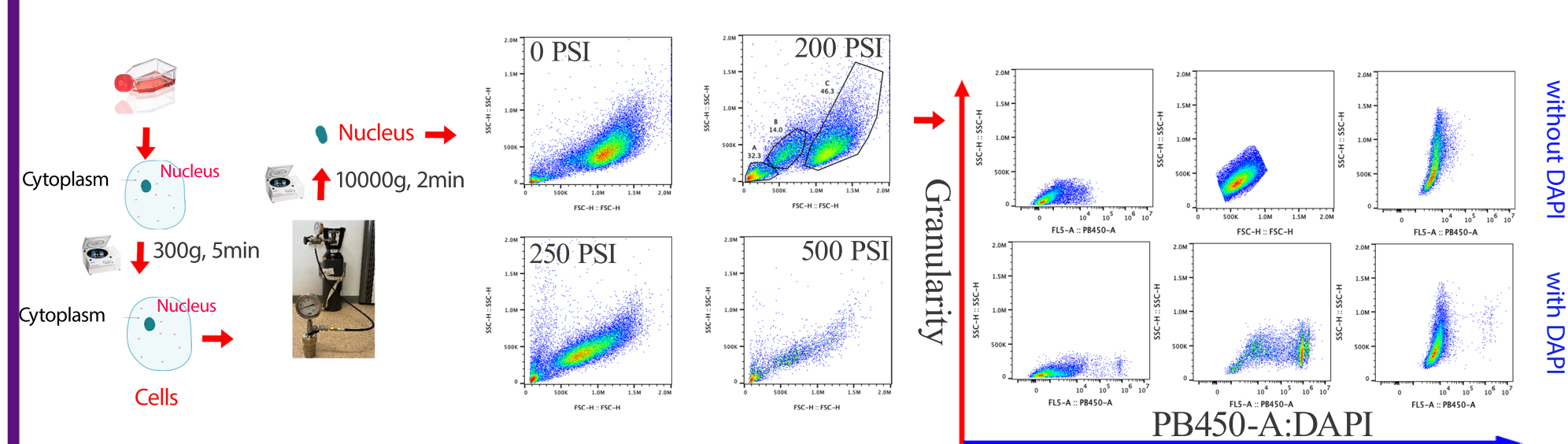


Fig 1: Schematic diagram of cavitation protocol for the isolation of nuclei from cell cultures. Nuclear localization was identified by DAPI staining in flow cytometry. 250PSI yields the highest number of intact nuclei.

Results

Nuclear accumulation of pPB-HSA requires an intact environment

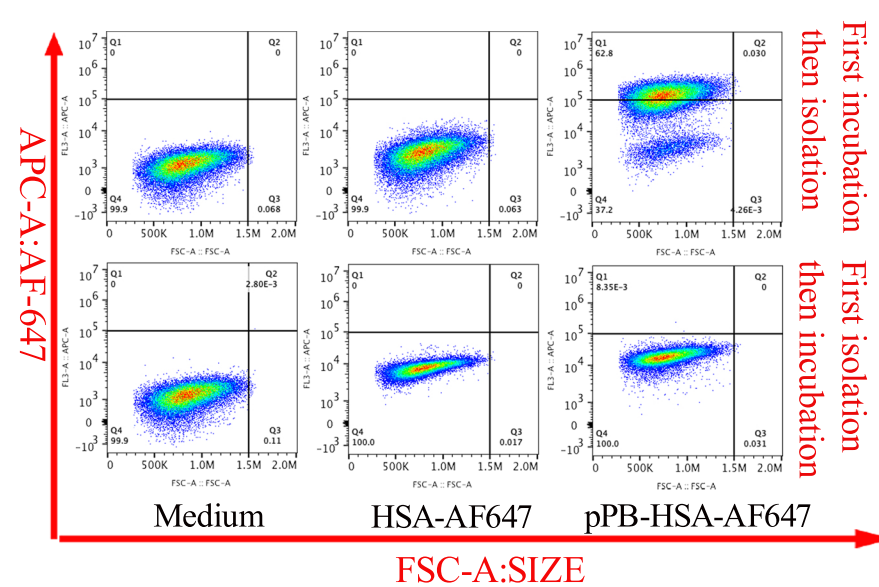


Fig 2: Binding of pPB-HSA before and after the cavitation: when pPB-HSA was added to nuclei after nuclear isolation, no nuclear binding is seen at all, while treatment of intact cells led to a significant nuclear accumulation of pPB-HSA.

Co-localization of pPB-HSA and PDGFR β in nuclei

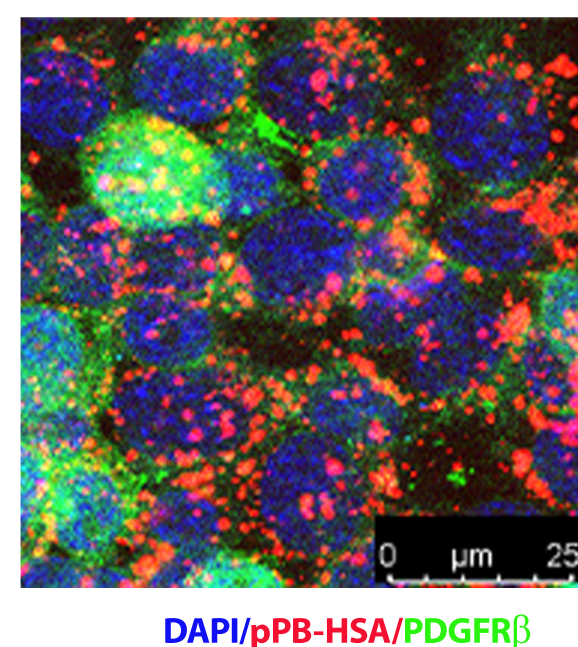


Fig 3: Confocal microscopy revealed colocalization of pPB-HSA (red) with nucleus (blue) and PDGFR β with pPB-HSA (red), as indicated by the pink and yellow signal in the merged image, respectively.

Co-localization of pPB-HSA and PDGFR- β in nuclei

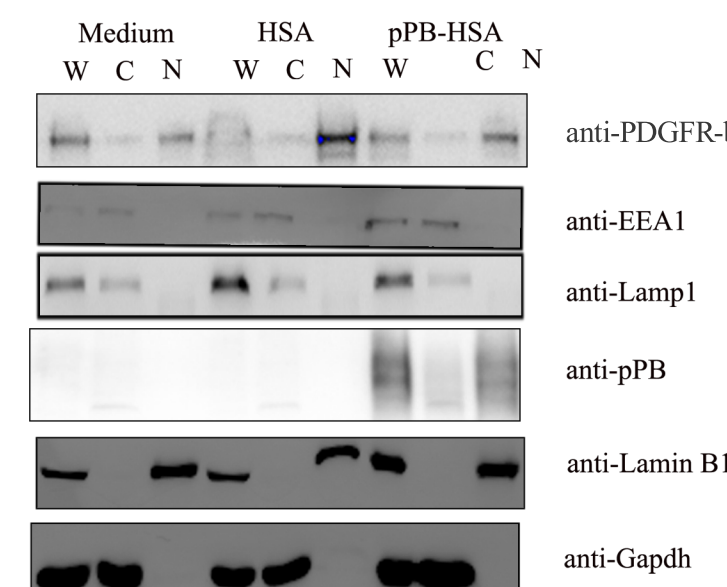


Fig 4: pPB co-localizes with PDGFR β in nuclei; Lysosomal markers are not in the nuclei. No contamination between nuclear and cytoplasmic fractions.

Visualization of pPB-HSA in nuclei by confocal microscopy

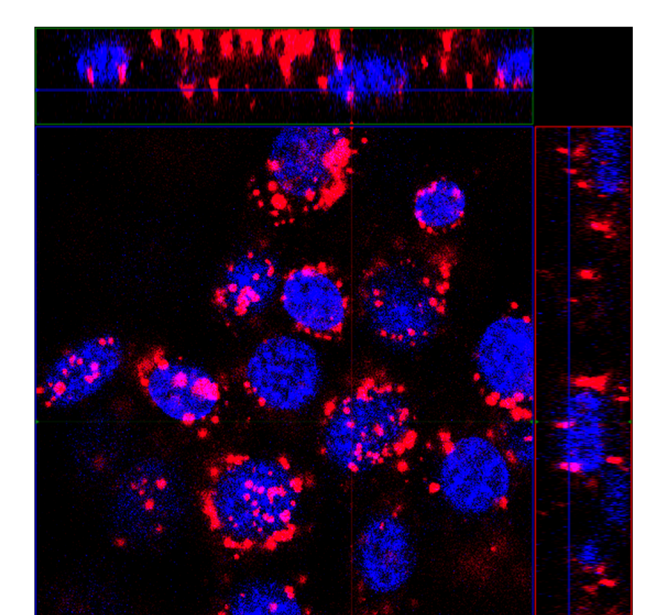


Fig 5: Orthogonal view of different planes (x/y, x/z and y/z) show pPB-albumin nuclear localization.

Enzyme intracellular activity assay

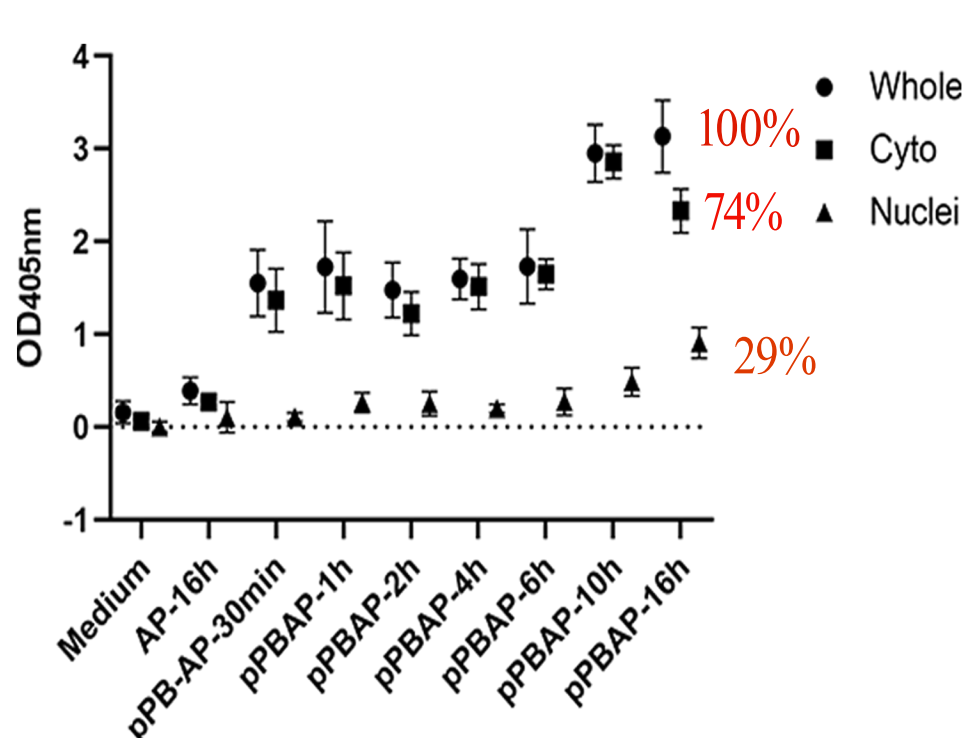


Fig 6: pPB-AP activity in different cellular fractions of over time; pPB-AP maintains its activity in nucleus, while AP is not taken up.

pPB-anti-NF- κ B-IgG Specific Blockade of NF- κ B

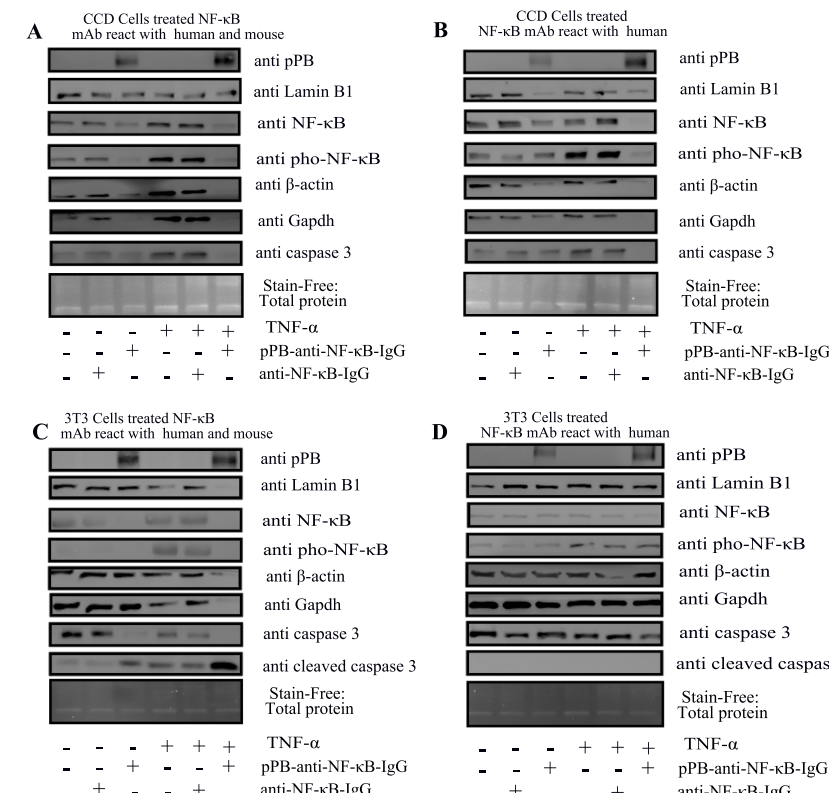


Fig 7: pPB-anti-NF- κ B-IgG mediated blockade occurs only in the context of species matching, and this blockade induces apoptosis.

ACTA2 silencing by pPB-Cas9+Sgrna(ACTA2)

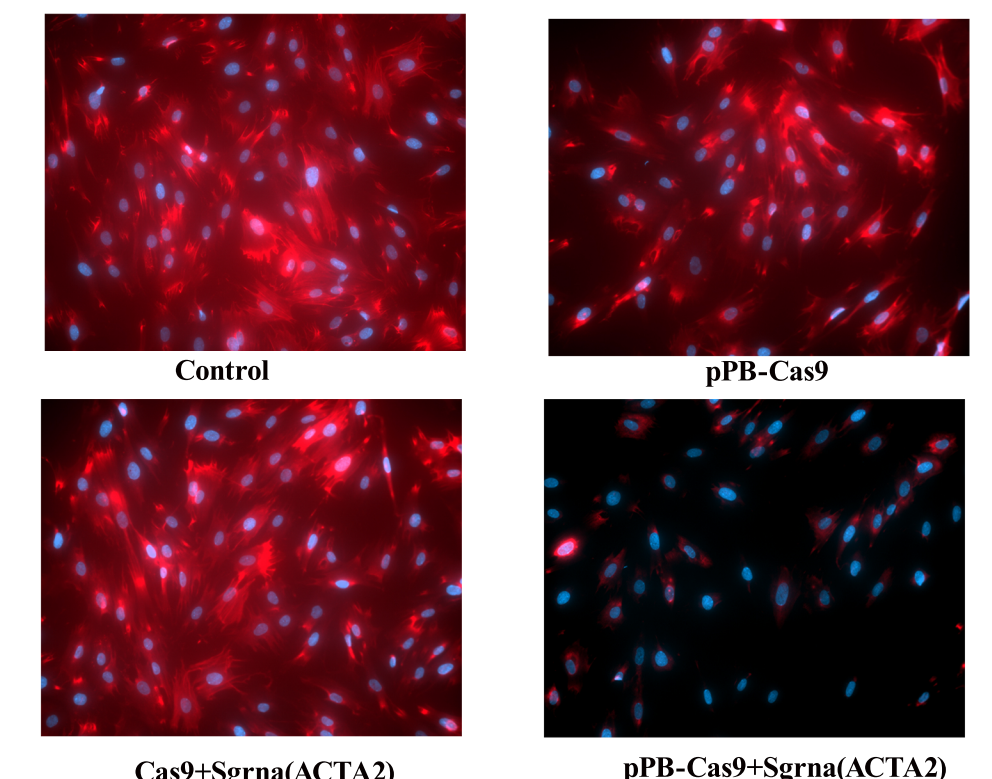


Fig 8: pPB-Cas9 + sgRNA(ACTA2) knocked out the ACTA2/ α -SMA protein; This knockout remains effective after four generations.

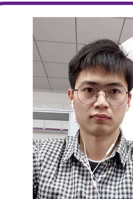
Conclusions: pPB-modified proteins bind to fibroblasts via PDGFR- β (Poelstra et al., 2023)

This complex is taken up by receptor-mediated endocytosis and delivered to the nucleus, similar to PDGFR β .

Proteins delivered to nuclear fractions are intact, and retain their functional activity.

pPB can be used as a versatile platform for the nuclear delivery of different biomacromolecules.

Acknowledgements:



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