Physico-Chemical Characterization of the Tumour Microenvironment of Pancreatic Ductal Adenocarcinoma

Alejandro Calderón-Villalba, Pilar Alamán-Diez, Elena García-Gareta, Jose Manuel García-Aznar

1 Multiscale in Mechanical & Biological Engineering Research Group, Aragon Institute of Engineering Research (I3A), Universidad de Zaragoza, Mariano Esquillor 6, 50018 Zaragoza, Aragon, Spain
2 Aragon Institute for Health Research (IIS Aragon), Miguel Servet University Hospital, Zaragoza, Aragon, Spain
3 Division of Biomaterials & Tissue Engineering, UCL Eastman Dental Institute, University College London, London, United Kingdom

Introduction

- Pancreatic ductal adenocarcinoma (PDAC) is the most prevalent form among all pancreatic cancer types.
- Limited response to conventional clinical treatments, which include chemotherapy, surgery and radiotherapy.
- PDAC displays a non-immunogenic, immune-suppressive and therapy-resistant microenvironment, largely attributed to its intricate tumor microenvironment (TME).

Aim and Experimental Design

- To characterize the physico-chemical properties of the PDAC tumor microenvironment using patient-derived xenografts (PDX) and advanced decellularization techniques. By understanding these properties, novel therapeutic strategies may be developed to target PDAC more effectively and improve patient outcome.

Results

1. Quantification of decellularization.

- FIG. 1 (A) Hematoxylin-eosin staining (magenta in pink and cells as blue dots) and (B) Alcian blue staining (GAGs in blue and cells in pink/purple dots) of native, partially decellularized and fully decellularized PDX samples.
- Histological stainings showed abundant desmplasia with extensive protein deposition recapitulating the pattern found in human PDAC TME. Alcian blue staining revealed the presence of GAGs in the ECM even after decellularization process. Scale bar = 100 μm

2. dsDNA quantification.

- FIG. 2 Quantification of double stranded DNA (dsDNA) was performed on DAPI stainings of histological sections showing a significant reduction in dsDNA content between the native TME, partially and fully decellularized. Residual blue fluorescence can be observed in the fully decellularized samples which may be indicative of the presence of cellular debris as well as residual dsDNA trapped in the ECM after decellularization.

3. Pore size distribution

- FIG. 3 Superficial pore size analysis based on SEM images of PDAC samples. (A) All pores, (B) Breakdown of pores in the 0 to 10 μm range of size. Our results showed that the decellularization released residual or accumulated stresses exerted by the cells to the TME, thereby opening the porous ECM network and increasing permeability to liquids, drugs, large molecules and cells used as therapeutic or delivery agents.

4. Collagen and GAGs quantification.

- FIG. 4 Collagen (A) and GAGs (B) content of native, partially and fully decellularized samples were measured using commercial kits. Quantification of total collagen showed a significant reduction in the amount of soluble collagen during decellularization process something expected since it is not heavily crosslinked to ECM. Insoluble collagen seems to increase in the decellularized samples however this is due to the normalization by wet weight. Regarding GAGs quantification, we observed a significant reduction with decellularization, which was expected since about 30% of GAGs are associated with the cell membrane and thus, is eliminated with the cellular fraction.

Conclusions

The incremental permeability and pore size observed during decellularization reveal that the cellular components of the TME exert a relevant contractile role in this particular tumor, probably due to a high presence of cancer associated fibroblasts (CAFs) which also contribute to the desmoplastic deposition of ECM proteins. This research offers a potential therapeutic strategy based on the application of therapeutic agents with small size such as small drugs or therapeutic cells and, although further work is required this opens a new innovative therapeutic field to develop new