Multi-omics strategy for the studies of immune regulation in pluripotent stem cell-based cardiac regenerative medicine in a swine model

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Introduction

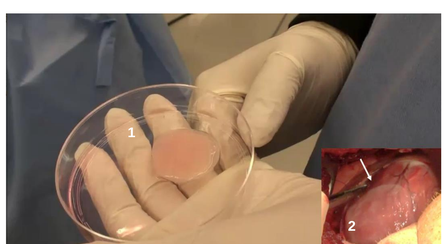
Human pluripotent stem cells (PSCs) derived cardiac tissue sheets is expected to be a complementary option for the treatment of intractable severe heart failure. Even though immune rejection rarely occurs when PSC-derived graft and the recipient have the same HLA type, it would not be sufficient to fully control immune rejection. Before going to a clinical trial, it is desirable to establish an appropriate protocol of administering immunosuppressive agents as well as MHC matching. As it is extremely important to identify biomarkers for the immune rejection, here we have established a swine allogeneic transplant models of myocardial tissue and heart sheets to analyze the rejection of PSC-derived cardiac tissue sheets from both tissue proteome and immune cell transcriptome.

Methods

Animals; Two micromini pigs with the same SLA were fed for the experiments with and without tacrolimus, mycophenolic acid and prednisolone for (A) and (B), respectively. The heart tissue sheets derived from porcine PSCs were then attached to the hearts of (A) and (B) (Fig. 1). After 1 week, the hearts were harvested by opening the chest.

Sample preparation; 10 μm frozen sections of hearts were cut on a cryostat (CM1950, Leica Microsystems, Wetzlar, Germany) and transferred to conductive Indium-Tin-Oxide (ITO) coated glass slides.

MALDI IMS (Fig. 2); On-tissue digestion with trypsin was performed with TM-Sprayer. α-cyano-4-hydroxycinnamic acid (CHCA) 10 mg/ml in 70% Acetonitrile was uniformly deposited on the slide by using TM-Sprayer(HTX Imaging). Then extracted peptides and proteins are measured by using timsTOF flex (Bruker Daltonik GmbH) with a spatial resolution of 100 μm. Ions were detected in mass range of m/z 700-4,000.



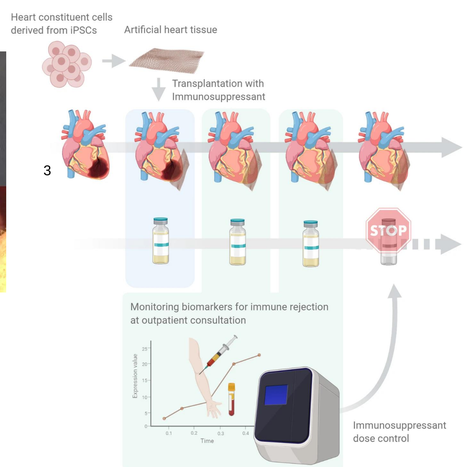


Fig. 1 PSC-derived cardiac tissue sheets
1. PSC-derived cardiac tissue sheets
2. PSC-derived cardiac tissue sheets were attached to the hearts of (A) and (B)
3. The system of detecting immune rejection

MALDI-IMS with shotgun proteomics (Fig. 2); Then extracted peptides and proteins are measured by using timsTOF flex (Bruker Daltonik GmbH) with a spatial resolution of 100 μm. On-tissue digestion with trypsin was performed with TM-Sprayer. By using tims TOF Pro with nanoElute (Bruker Daltonik GmbH), shotgun proteomics was performed with the same tissue sample. Column used was 25 cm × 75 μm, C18 column.

Data Analysis; Obtained mass spectra as well as annotated proteins and peptides were visualized with flexiImaging and SCiLS Lab 2022b Software.

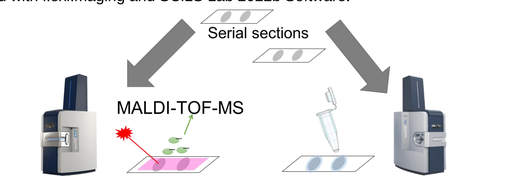


Fig. 2 Workflow image of combined analytical method

Results

① Histological Analysis

Histopathological findings of transplanted tissue sheets from (A) with immunosuppressive agents increased sheet thickness, whereas transplanted tissue sheets from (B) without immunosuppressive agents showed no thickness alterations. Between the myocardium and PSC-derived cardiac tissue sheets, a massive eosinophilic infiltrations were observed in the tissue of (A), while apparent inflammatory infiltrations were not observed in the tissue of (B) (Fig. 3).

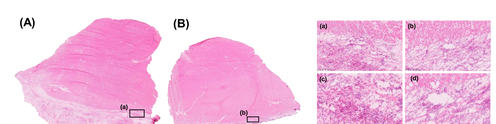


Fig. 3 HE staining of the heart from two micromini pigs. (A) - (B); bar=5mm. (a) – (b); bar=250μm (c) – (d); bar=100μm (A); Micromini pig (A) with tacrolimus, mycophenolic acid and prednisolone (B); Micromini pig (B) without tacrolimus, mycophenolic acid and prednisolone (a); Eosinophilic infiltrations were observed (b); No apparent inflammatory infiltrations were observed (c); Enlarged view from (a) (d); Enlarged view from (b)

② MALDI-IMS

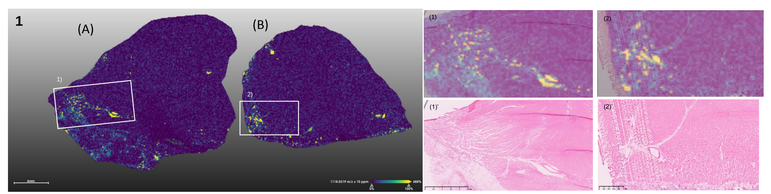
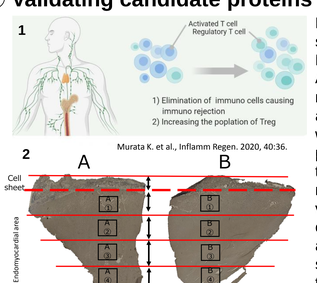


Fig. 4 **The image of the discriminative single peak and specific histological features of the pig heart.** 1.The image of the single peak at *m/z* 1118.0319. 1) and 2); Selected areas of profound signal intensity in (A) and (B) respectively. (1) and (2); The single peak image registered with HE staining image which is enlarged from 1) and 2) respectively. (1)' and (2)'; Enlarged view HE staining image from 1) and 2) respectively.

③ Validating candidate proteins in multi-omics strategy



Prior studies have indicated that stem cell-derived cell sheets improve cardiac function, for example, in Myocardial Infarction (MI), mainly via paracrine action. As a candidate protein/peptides in ② was localized mostly in cell sheets in a diffuse manner as well as intra- and peri-vascular area of endomyocardial area along with vascular structure (Fig. 5 - 2). This suggests that a protein/peptides is highly plausible candidate because transplanted cell sheet can induce host tissues to neovascularize via secreting cytokines. For further validation study, circulating immune cells can be a target of multi-omics study including transcriptome of CD3 and/or CD8 positive T cell fraction (Fig. 5 – 1). Now several candidate proteins were also challenged for both tissue based immunohistochemistry as well as immune cell based analysis.

Fig. 5 1. Mechanisms of immunosuppression induced by Treg. 2. The image of immunohistochemical staining of a candidate protein/peptides. Dividing it into four areas from the endocardium to the cell sheet attachment area

Conclusions

Through tissue proteomic imaging with shotgun proteomics and T cell transcriptome, a candidate protein/peptides is now in a validation step for further studies of cardiac cell sheet rejection.