Stable PET-reporter gene transfection of MSCs for in vivo long-term cell tracking in xenogeneic transplanted tissue engineered heart valves.

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Introduction

Transient cell transfection with plasmid vectors is regarded as safe method in human gene therapy, since it is free of the risk to permanently introduce genetic material into the host cells genome. As part of the LifeValve EU project it was our aim to transfect porcine mesenchymal stem cells (MSCs) with a positron emission tomography (PET)-reporter gene, seed the cells to tissue engineered heart valves (TEHV) and implant them into sheep (xenogeneic transplantation). The fate of the transiently transfected stem cells should be tracked via serial in vivo positron emission tomography-computer tomography (PET-CT).

Methods.

Porcine MSCs were transiently transfected with HSV-1 truncated tyrosine kinase PET-reporter gene using Lipofectamine reagent as a carrier. Cells were seeded in a concentration of 7×10^6 cells onto each TEHV, which were then percutaneously implanted to the pulmonary valve position of sheep (n=8). Serial PET-CT imaging of implanted valves was conducted after 3h, 24h, 3 weeks and 6 months using mCi [18]F-FHBG PET tracer that specifically binds to HSV1-tk PET-reporter gene. For the quantification of cells in the TEHV in vitro dilution series of PET-MSCs mixed with PET-tracer were PET-CT imaged.

Results

Depending on the cell number of transient transfected PET-MSCs the corresponding tracer uptake could be observed in the PET-CT images of cell dilution series. There was an accumulation of seeded cells observed at the base of the leaflets of the TEHV that were in vitro tested with PET-CT. Implanted PET-MSC TEHV showed a clear PET signal after 3h (calculated cell number 4.95×10^6) and there was no significant decrease of living cells at 24h and 3 weeks after valve implantation (estimated cell number 4.67×10^6). Interestingly, PET-CT images after 6 months depicted a clear PET signal (estimated 3.16×10^6 cells) on the valves that are indicating a spontaneous stable transfection with PET-reporter gene.

Conclusion

Serial in vivo long-term tracking of xenogeneic MSCs seeded to TEHVs that were implanted into sheep could be reported for the first time. Long-term follow-up revealed spontaneous stable transfection of the PET-reporter gene, which suggests the risk of genomic mutation induced by plasmids. The study was supported by the LifeValve EU project.
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