Mesenchymal Stem Cells (MSC) and Angiogenesis in Pancreatic and Prostate Cancer

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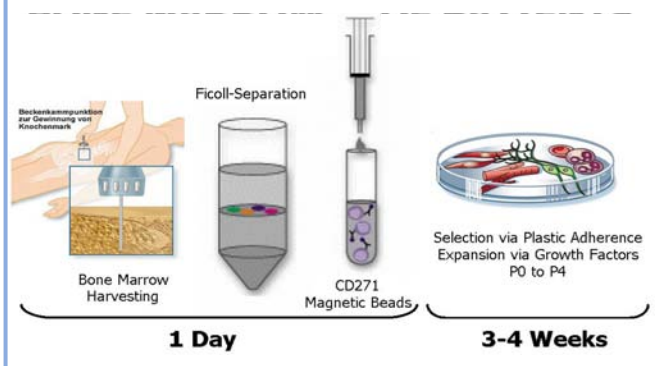
Introduction

Homing of manipulated mesenchymal stem cells (MSCs) to glioma xenograft models has been demonstrated in recent reports. MSCs may contribute to the formation of tumour stroma and tumour blood vessels and thus may be suitable vehicles for the targeted transfer of therapeutic genes.
The formation and maintenance of blood vessels in tumours is controlled to high extend by VEGF and its receptors (-RI, -RII). In parallel, the transcription factor Hypoxia-Inducible Factor-1 (HIF-1), which is activated specifically in the tumour micro-environment, is regulating the expression of VEGF and VEGF-RI. Thus, a blockade of HIF-1 and VEGF in tumour infiltrating MSCs might interfere with the homeostasis of tumour vascularisation.
In this work we could stably transduced MSC with lentiviral vectors and could design functional anti-angiogenic siRNAs.

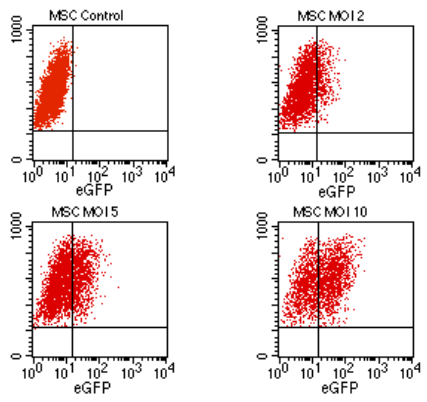
Results

Purification of MSCs from bone marrow of patients



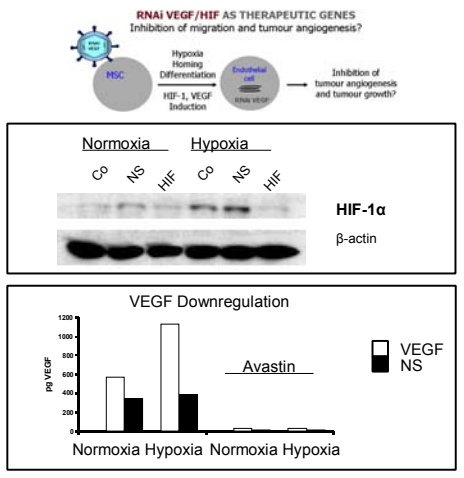
MSCs were isolated by Ficoll centrifugation and CD271 MACS separation. Then they were cultured in specific MSC medium.

Lentiviral transduction of MSCs



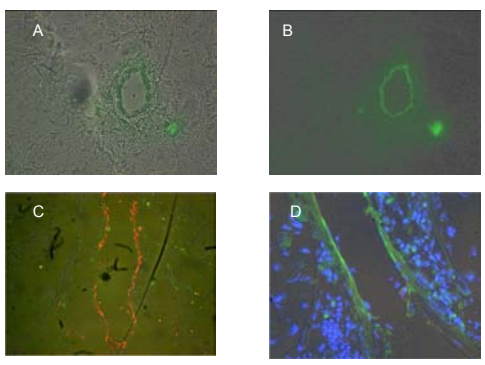
Human MSC generated *in vitro* can efficiently be transduced using lentiviral vectors.

Lentiviral RNAi downregulation



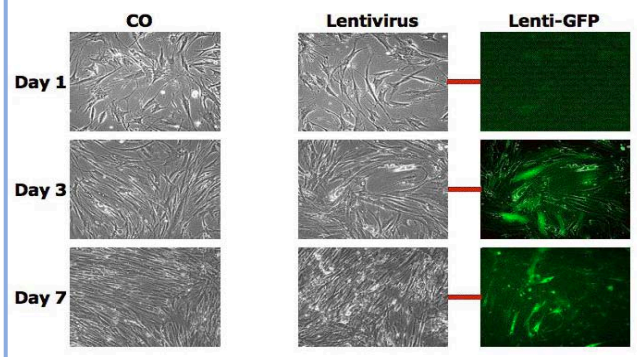
Transduction of BxPC3 cells with lentiviral HIF-1 and VEGF RNAi constructs efficiently silenced protein and mRNA expression, respectively.

Transdifferentiation of MSC into Endothelial Cells ?



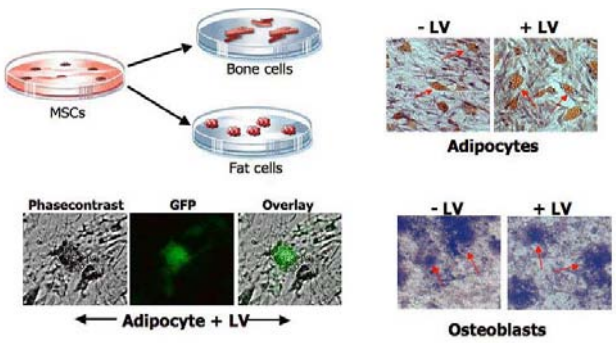
GFP labelled MSC were injected in the tail vein of an orthotopic pancreas tumour bearing nude mouse and organs shock frozen. A) Phase contrast and Green fluorescence overlay of tumour section. B)Unstained GFP fluorescence image of A. C) Tumour section stained for GFP and vWF D) tumour section stained for GFP and with DAPI

Transduction does not alter MSC phenotype



MSCs maintain their phenotype after lentiviral transduction.

Differentiation of MSCs was not altered by lentivirus



MSCs maintain the potential to differentiate into adipocytes (Oil-Red-O staining) and osteoblasts (van Kossa staining) following lentiviral transduction.

Summary

- **We could show that it is possible to stably transduce MSCs using lentiviral vectors.**
- **The designed siRNAs were fully functional in *in vitro* assays (ELISA and Western Blot), shown by the down regulation of VEGF and HIF-1, respectively.**

Outlook

To facilitate *in vitro* tests, the evaluation of a lentiviral plasmid carrying the siRNA together with a GFP puromycine fusion protein as selectable marker is currently in progress.
To further evaluate the siRNA function, we next test the interaction of transduced MSC and endothelial cells in a spheroid assay.
To truly test the anti-angiogenic activity, the use of transduced MSCs in a CAM-assay as primitive *in vivo* system is envisaged.
Transdifferentiation of homed MSCs into the tumour will become a focus.