Spatio-temporal expression control of a heat shock promoter-driven transgene delivered in the kidney by modified mesenchymal stem cells: A feasibility study using MR guided Focused Ultrasound

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Introduction

Genetically modified stem can be modified to express a transgene (1). Spatiotemporal control of expression is required for most gene therapy procedures. Heat-shock promoters (HSP) have been shown to be good candidates as inducible promoters allowing a noninvasive spatial and temporal control of gene expression when combined with noninvasive heating by focused ultrasound (FUS) (2).

Materials and Methods

Mesenchymal stem cells (MSC) from bone marrow of Lewis 1A rats were transfected with HSP-Luc expressing the luciferase gene under control of a HSP-70 promoter. The phenotype and the differentiation properties of the obtained HSP-luc MSC clone were assayed. For in vitro studies, cells were exposed to various induction temperatures during 20 min. For in vivo studies, rats (n=4) were anesthetized, and 300000 MSC cells were injected in the left renal artery. The animals were installed in a plastic tube to reduce motion artefacts. During the FUS heating procedure, MR temperature mapping was used for feedback control of the deposited thermal energy (3). Respiratory-gated, fat-suppressed, Gradient Echo Echo-Planar Imaging (GE-EPI) sequences were used for MR temperature maps with one image (96 mm FOV, acquisition matrix 128 x 96, voxel size 0.75 x 0.75 x 5 mm) during each respiratory cycle. Animals were sacrificed 8 to 12 hours after the heating procedure. To assess MSC localization and luciferase expression, frozen serial microsections were performed.

Results

Cloned HSP-luc MSC cell lines retained MSC phenotype and ability to differentiate towards adipocyte or osteogenic lineages. *In vitro* luciferase transgene expression showed an optimal temperature of 45°C for induction of the luciferase gene for a 20 minutes heat shock. A peak of luciferase concentration was seen 12 hours after heating with a return to background expression by 48-72 hours.

Figure 1 shows the experimental setup, a temperature map, and the heating curve for the in vivo experiments. Standard deviation of the measured temperature in steady state regimen (45°C during 5 minutes) ranged between 1.5°C and 2°C. The injected renal territory was visualized by temporary bleaching upon intraarterial injection of MSC cells. The injected pole was heated using MRI-controlled FUS hyperthermia. Results in each animal showed expression of the luciferase in the kidney parenchyma that had been grafted and heated (Figure 3). Only background luciferase activity was observed in the non injected territories.

Discussion

These results demonstrate the feasibility of in vivo induction of transgene expression in intrarenally delivered modified MSCs by MRI-controlled FUS hyperthermia. Non-heated areas where MSCs were detected showed a low level of expression of luciferase presumably due to stress unrelated to the heat-shock.

References

1) Baksh et al J CELL MOL MED 2004;8:301-316.; 2) Salomir et al. RM 2000;43:342-347.3) Guilhon et al. J GENE MED 2003;5:333-342.

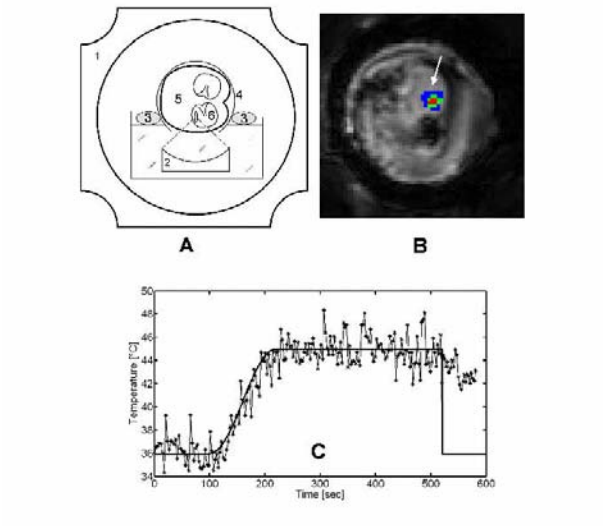

Figure 1. MRI-controlled FUS in the kidney.
A. Schematic representation of the experimental setup: 1. magnet, 2. FUS transducer, 3. MR receiver coil, 4. plastic tube, 5. rat and 6. kidney. **B.** Example of MR temperature map (blue 39°C, green 42°C and red 45°C). **C.** Temperature time course in voxel containing the focal point. The asterisks indicate MR obtained thermometry data.

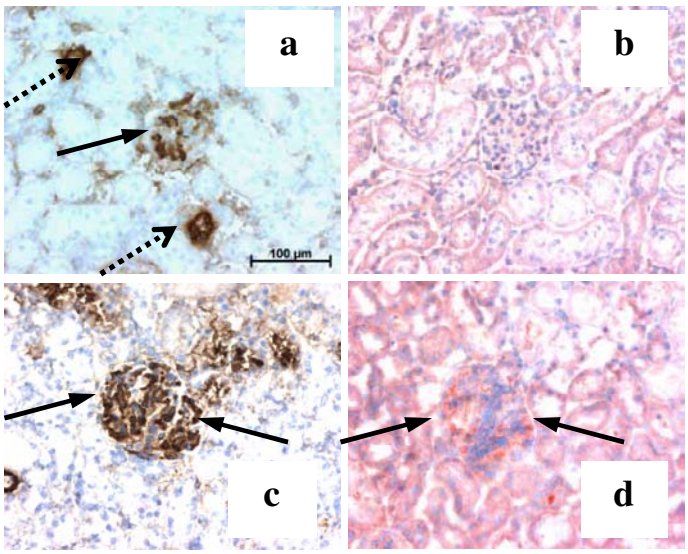

Figure 2: (a,b) Non-heated kidney with (a) SMA positive MSCs in glomeruli (plain arrows). SMA staining (brown) is also seen in vascular smooth muscle cells (dashed arrows). (b) No luciferase expression (red) is detectable. Heated kidney sections with MSCs (c,d) show SMA positive MSCs in glomeruli (c, plain arrow) but only MSCs in glomeruli are positive for luciferase (d, plain arrow).