Non-invasive spatial control of gene activation by local heating with focused ultrasound under MRI temperature guidance

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Introduction

Gene therapy is a promising tool for the treatment of a wide variety of diseases including cancer, inherited genetic disorders and neurodegenerative disorders. However, several drawbacks must still be overcome before gene therapy can be found in the clinical environment. Besides the problem of low efficiency of gene delivery in vivo, spatial and temporal control of the gene expression are major challenges in this domain. The possibility of controlling transgene expression with localized heating in a tumor using a heat shock protein promoter and MRI-guided focused ultrasound (FUS) has been shown previously [1]. The aim of this study is to: 1) Investigate the spatial correlation between the region where the transgene is activated (i.e. temperature increase) and the region where the transgene product is found. 2) Study whether the promoter is activated by heat only or whether ultrasound induced mechanical effects may also lead to gene activation.

Materials and Methods

MRI imaging: Dynamic MR temperature imaging was performed on Philips Achieva 1.5 Tesla with a segmented EPI sequence (EPI-factor = 5, TE = 15ms, TR = 34ms, flip angle = 16^0, FOV = 64 × 56 mm^2, matrix 64 × 64 matrix, 3 slices) with a 23 mm surface coil positioned above the mouse hind leg.

Focused Ultrasound System: Heating was performed with an in-house designed single channel focused ultrasound transducer incorporated in the bed of the MR system. The dimensions of the focal point are 1 × 1 × 4 mm^3.

Animal model: Transgenic mice (NFL1, n = 3), containing a transgene that allows firefly luciferase expression under control of the heat shock protein 1B (Hspa-1b) promoter, were used in this study [2].

BLI imaging: Bioluminescence images were sequentially acquired with the NightOWL LB 981 NC 100 CCD camera (Berthold Technologies) to measure luciferase activity. Two minutes before sedating the animal with isoflurane (2%), 100 µl of an aqueous solution of luciferin (29 mg/ml stock) was injected into its peritoneal cavity (ip). Bioluminescence images (2 accumulations of 1 minute, 2×2 binning) were taken 7 and 10.5 minutes after the luciferin injection into the animal in prone and supine positions, respectively. Grey-scale body-surface reference images were also collected in both positions. A pseudocolor luminescent image, representing the spatial distribution of the detected photons emitted from active luciferase within the animal, was generated using WinLight software (Berthold Technologies). Signal quantification was done by calculating the average number of photons/s/pixel above a threshold value of 200 photons/s/pix inside a region of interest.

Heating protocol: The animals (n = 2) were sedated with isoflurane (2%) and the treated (left) hind leg was injected with muscle relaxants (100 µl lidocaine (2.5 mg/ml) at 3 positions intra muscularly and 200 µl ropivacaine (0.5 mg/ml) at two positions subcutaneously) and with 600 µl ketamine (1.67 mg/ml) as analgesic (t = 0). The animal temperature was kept constant at ± 35^0 C with a heating plate. At t = 10-15 minutes the animal was positioned into the MRI, above the focused ultrasound transducer. The animal temperature was kept constant by a heated water bath surrounding the animal, which also provided an air-bubble free interface between the transducer and the animal. At t = 30 minutes, heating was started with a protocol whereby the temperature was kept constant at 43^0 C for 8 minutes. Temperature was controlled at the focal point by automatic feedback of the output power delivered to the focused ultrasound transducer [3]. Finally, BL imaging was done at t = 4, 6, 7 or 9 and 24 hours. The potential effect of mechanical gene activation by FUS was also investigated in the present study. For that purpose, identical total amount of energy was applied on a different mouse with a pulsed FUS power deposition (20% duty cycle, 5 times longer duration) to avoid tissue heating. Temperature images were acquired to verify that no temperature increased was induced.

Results and Discussion

Standard deviation on temperature control was 1^0 C. Figure 1 shows the MR temperature maps (A and C, 5 minutes after the beginning of the heating protocol) and the bioluminescence images (B and D) 6 hours after heating in two mice. As seen from image A and C, applying the same heating protocol does not lead to identical temperature distribution in the heated region, which can result from local conduction differences between animals. These differences also clearly appear on BLI images (B and D), indicating that a close correlation exists between the local temperature distribution and the region where luciferase activity (i.e. light production) is found. Figure 2 shows the kinetics of the light production linked to luciferase activity after heating. A maximal activity was found 6-7 hours after heating. The total applied energy during the heating protocol was 771 J and 876 J for the two mice. BL image of the control mouse (840 J in pulsed FUS) displayed a very low intensity (< 400 photons/s/pix) and diffuse light production at 6 hours. For this experiment, no significant temperature increase (less than 1^0 C) was observed.

Conclusion and Perspective

This study demonstrates that local activation of genes by MRI controlled focused ultrasound heating at 43^0 C is a powerful tool for spatial control of gene expression. This physical method provides a high spatial precision since location and sizes of the heated regions correspond to the region producing light. Local gene activation was mainly dependent on temperature and not to mechanical stress of the FUS on the tissues. However, this remaining low level light production could be due to several sources of stress (low level mechanical stress, heated water bath, shaving, etc…) and need further investigations to provide a quantitative analysis. We demonstrate here that local gene expression using the Hspa-1b promoter can be precisely controlled by combining non-invasive FUS heating under precise MRI temperature guidance. Despite no visible damage was observed on the skin of the heated legs, additional histology experiments should be performed to confirm the non invasive nature of the complete procedure.

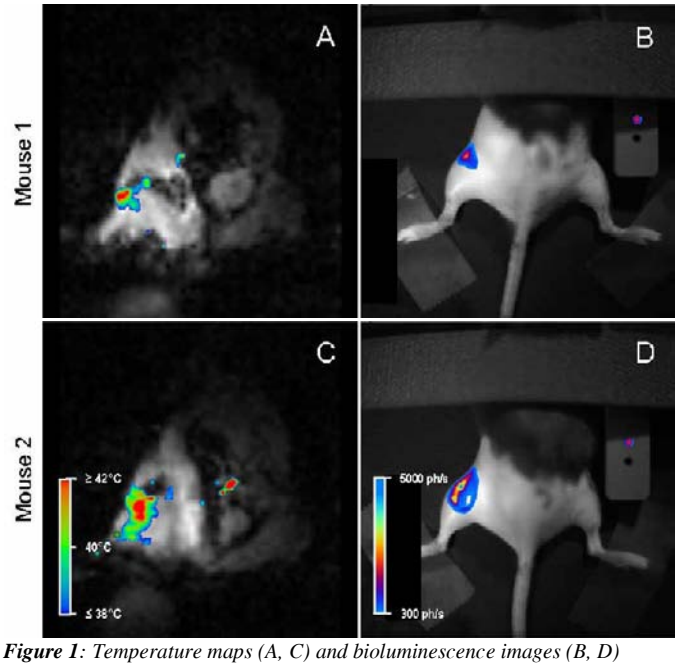

***Figure 1**: Temperature maps (A, C) and bioluminescence images (B, D) acquired 6 hours after heating in 2 mice.*

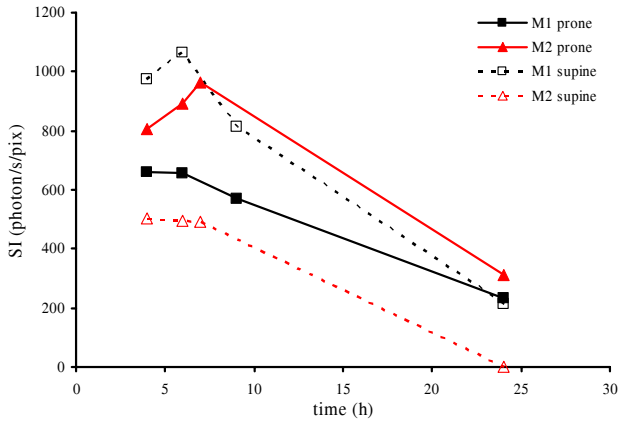

***Figure 2**: Kinetics of light production after heating linked to luciferase activity for 2 mice. Solid and dashed lines represent light intensity measured in prone and supine positions, respectively.*

References

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