

Imaging of tumor angiogenesis in a novel skin chamber using MRI and optical imaging

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Introduction

Angiogenesis is an important process in the pathogenesis of tumors, and is thus a target for the development of novel approaches for cancer therapy. Non-invasive imaging methods can indicate changes in vessel morphology and function including tumor perfusion and permeability. Different imaging methods are used to assess vascularization, in particular MRI and optical imaging. Due to the complementary character of MRI and optical imaging, it is highly desirable to study angiogenesis with both imaging techniques simultaneously. The aim of our study was to design a skin chamber, in which tumor vascularization could be imaged non-invasively with both, MRI and optical imaging in the same tumor model.

Materials and Methods

An MR-compatible skin chamber for optical imaging was designed consisting of a teflon ring and an optical window (Figure 1; a variation of this skin chamber was described before by Bock M et al, ISMRM 2008). Around the skin chamber, a dedicated external loop RF coil was placed during each measurement to maximize the SNR from the chamber interior.

The skin chambers were implanted in 5 nude mice, and high-grade malignant human squamous carcinoma HaCaT-ras A5RT3 cells were seeded into the chamber. Three weeks after tumor cell inoculation, optical imaging and MRI were performed. For MR imaging, high resolution images were acquired on a 1.5T scanner (Magnetom Symphony, Siemens) with a 3D FLASH sequence before and after administration of an intravascular contrast agent (0.1 mmol per kg; Vasovist, Schering) with the following parameters: TR = 14.3 ms, TE = 5.8 ms, flip angle = 35°, FOV = 61×80 mm², averages: 1, slice thickness: 140 μm; acquisition time: 8 min 34 s). In addition, dynamic contrast-enhanced MRI (DCE-MRI) was performed (turboFLASH, TR = 18 ms, TE = 6.7 ms, flip angle = 12°, FOV = 40×50 mm², averages: 2, slice thickness: 2 mm, acquisition time: 5 min 14 s). Images of the tumor vasculature were obtained after subtraction of the pre- from the post-contrast images, and a maximum intensity projection (MIP) was used to delineate the vasculature. Semiquantitative parameters including *peak enhancement* were calculated in the tumor and expressed as color-coded maps (Dyna Lab, Mevis Research, Germany). Optical fluorescence imaging was performed using a Leica MZ 16 FA microscope acquiring images before and after i.v. application of FITC (0.1 μg FITC per kg; Fluorescein-isothiocyanate-Dextran 150000-Conjugate, Sigma). After MRI and optical imaging, the animals were sacrificed for immunofluorescent analysis of the tumor using CD 31 and DAPI staining.

Results and Discussion

HaCaT-ras A5RT3 proliferated in the skin chamber and infiltrated the host tissue. A well-defined tumor was visible approximately 3 weeks after tumor cell inoculation. In the skin chamber, host-derived vessels that penetrated into the tumor tissue could clearly be visualized (arrows, Figure 2). With optical imaging, the tumor could be clearly delineated in the skin chamber through the optical window, but individual tumor vessels could not be confined (Figure 3A). In the *peak enhancement* maps (Figure 3B) a heterogeneous distribution of high and low values throughout the tumor was found. When comparing the color maps of *peak enhancement* with fluorescent analysis (Figure 3 C), corresponding areas of high and low vascularized tumor parts (Figure 3 B, C) were seen.

In a novel skin chamber tumor vascularization could be analyzed non-invasively in nude mice using MRI and optical imaging in the same setting. This chamber facilitates research on the pathogenesis of angiogenesis and the assessment of treatment response.

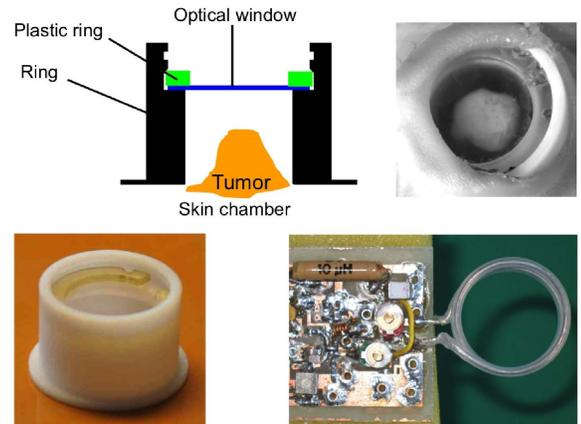


Figure 1. Schematic illustration of the MR-compatible skin chamber for simultaneous optical imaging (top left). The optical window is fixed in the chamber with a removable plastic ring (bottom left). After implantation (top right) tumor cells grow in the interior of the chamber. For high-resolution MR imaging an external loop coil (bottom right) is placed over the chamber.

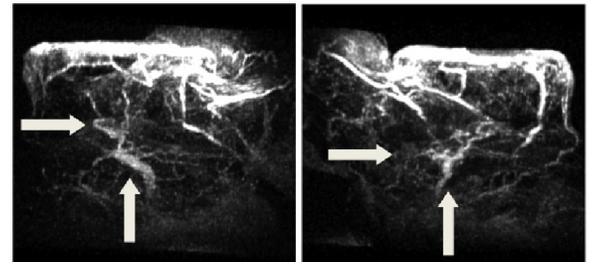


Figure 2. 3D MIP of the tumor vasculature as acquired by MRI. Arrows point at host-derived vessels supplying the tumor tissue.

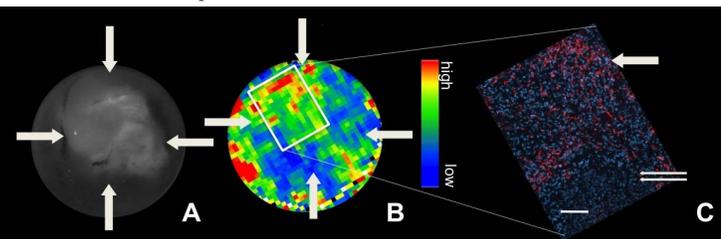


Figure 3. Microscopic fluorescence image of the tumor (arrows) after application of FITC (A) together with the color-coded *peak enhancement* map (B). Fluorescence analysis (C) of the marked tumor area including blood vessels (red: CD-31 staining for endothelial cells) as well as tumor and stroma cells (blue: DAPI staining for cell nuclei). Single arrow points at highly vascularized tumor tissue, double arrow indicates an area with reduced vascularization. (C; white bar: 100μm).