Imaging method for model-based control of tumor diseases

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***Abstract*—Modern approaches of cancer therapies have specific effect on the typical mechanisms of uncontrollably growing and multiplying tumor cells. These targeted therapies can be more efficient than commonly applied methods in clinical practice. Antiangiogenic therapy prevents tumors from forming new blood vessels to ensure sufficient oxygen and nutrients. Without appropriate vascularization, the development of the tumor is inhibited, thus metastasis formation is blocked. In this paper we briefly review the importance of antiangiogenic therapy. To improve mathematical models of tumor growth under angiogenic inhibitors, the process of tumor development must be analyzed. We have chosen Magnetic Resonance Imaging (MRI) to follow up the dynamics of tumor growth and monitor the effect of angiogenic inhibitors. T1-weighted images have been acquired using gradient-echo, spin echo and fast spin echo sequences to examine subcutaneous mouse tumors. We have found that fast spin echo results the best solution: short data acquisition time and good contrast without contrast agent.**

I. INTRODUCTION

Permanent changes in the DNA sequence may result in cancer cells, which can grow and divide in an unregulated way [1]. Tumor cells possess a wide variety of characteristics, which is utilized by the modern approaches of cancer therapies [2]. One of these specificities is the ability to inducing angiogenesis, i.e. forming new blood vessels to get more oxygen and nutrients from blood [3]. In the case of solid tumors, while the diameter of the tumor is less than approximately 1-2 mm, diffusion from surrounding tissue ensures sufficient oxygen and nutrients for tumor cells. When the tumor exceeds this limit, the oxygen and nutrient concentration decrease toward the core [4]. In order to further growth an angiogenic switch is required. In this stage the tumor becomes capable to create own blood vessels [5]. When tumor cells start to secrete angiogenic growth factors, they perturb the well-controlled balance of pro- and anti-angiogenic factors [6].

Antiangiogenic therapy is a new and unorthodox treatment and its goal is to prevent this process and keep the tumor in a certain state [7], [8], [9]. Without appropriate vascularization, the development of the tumor is inhibited, thus metastasis formation is blocked [10]. Compared to the most commonly used treatments in clinical practice (e.g. surgery, chemotherapy and radiation therapy), the antiangiogenic therapy has targeted effect on a specific mechanism [11]. Not only cancer cells are able to create new blood vessels, however angiogenesis takes place in the human body only under well-defined circumstances [12]. In adults angiogenesis occurs in the case of regeneration after injury, low oxygen concentration due to high altitude and during given stages of the menstrual cycle in women. Antiangiogenic therapy can be more effective than classical anticancer treatments, while the occurrence of side effects decreases.

In order to further development of the therapy, the process of the tumor growth must be analyzed and points of effective intervention must be located [13].

The dynamics of the tumor growth can be traced using non-invasive imaging methods. Magnetic Resonance Imaging (MRI) is a very effective in vivo imaging technique to visualize the internal structure of the body in detail, applying the nuclear magnetic resonance phenomena [14]. MRI provides excellent soft-tissue contrast, which makes it particularly useful in tumor imaging [15].

In research tasks, MRI can help us to observe the process of tumor growth in an objective way, without physiological intervention. Furthermore, the effects of angiogenic inhibitors can be monitored by MRI, which is important to create and validate mathematical models.

The paper is organized as follows. In Section II, we review the principles of MRI, focusing on small-animal MRI and present the main differences. In Section III, we introduce the benefits of tumor imaging applying MRI and then some results of tumor imaging studies are demonstrated. In Section IV, we present our experiment's results of subcutaneous mice tumors with small-animal MRI. The paper ends with the conclusion in Section V.

II. APPLICATIONS OF MAGNETIC RESONANCE IMAGING

A. Basics of MRI

MRI provides highly detailed images of the body, based on the interaction between hydrogen nuclei and extremely powerful external magnetic field. Hydrogen nuclei, i.e. protons have special quantum-mechanical properties called spin. Due to the magnetic characteristic of protons, the signals which are collected to create an image are related to the proton density of the material. Essentially, MRI creates the possibility to differentiate between tissues based on their water content and material dependent relaxation time constants that originates from their molecular structure [16].

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B. Small-animal MRI

The structure of small-animal MRI scanner is very similar to the typical human tunnel MRI scanners, which are frequently used in clinical practice. The main component of MRI equipment is a magnet (mostly superconducting electromagnet), which creates a strong magnetic field. Its inhomogeneities are eliminated by shim coils. In addition, the scanner consists of gradient coils, radio frequency and receiver coils. The image reconstruction and processing are done by computer [17].

The most significant difference between small-animal and human MRI scanners is the diameter of the patient table. In the case of small-animal MRI, it is scaled down to study mice and rats. The smaller diameter results in much stronger magnetic field and more homogeneous field. The quality of the image produced by the scanner depends on the power of the magnetic field applied during the measurement. Small-animal MRI devices, which field strength is usually in the range of [4.7, 14.1] Tesla can create more detailed and informative anatomical images. The spatial resolution can be less than 100 micrometers and the time of examination is shorter due to the greater signal-to-noise ration. The principle of measurement and the sequences used are essentially the same as the ones applied during the examination of human subjects.

Compared to human MRI, small-animal MRI is characterized by much better reproducibility, thus it produces more information in the given circumstances. It allows scientists to test lesser-known or potentially lethal chemical agents and drug interventions. Nowadays major problems arise from the objective comparison of the results of animal and human studies. MRI - as an important tool of translational medicine - can help to match animal models and human disease in pharmacological research. The most critical step is selecting an appropriate animal model in case of neuropsychiatric disorders (e.g. schizophrenia, autism, depression, bipolar disorder). By MRI images we can investigate the morphological changes of structures and the various effects of agents. Since both human and animal studies can be performed with this method, well functioning animal models can produce the same results as in the case of human patients. Therefore, MRI allows the validation of models of a number of diseases [18].

The examination of mice and rats is accomplished by using anesthesia; hence less movement artifact correction is needed in the phase of image processing.

III. MRI FOR IMAGING TUMORS

A. Commonly used methods in clinical practice

Particularly good contrast can be achieved using MRI when visualizing soft-tissues and lesions, which would be hidden by bone shadows in a radiograph. One of the many great advantages of this imaging technique is that it provides information about the function of the examined organ in addition to its structure.

Today the early detection of tumors is emphasized. In some cases whole-body-scan is recommended in order to search for tumors. With the help of the MRI tumor size and location can be both determined, furthermore it can be decided whether or not the tumor cells can spread to other parts of the body through the vascular or lymphatic system. During treatment, monitoring of tumor size and early recognition of metastasis formation are also practical.

Contrast-enhanced axial and coronal images show pathologic processes occurring in the brain such as tumors, inflammations and infections, which damage the blood-brain barrier, thus T1-weighted images become bright. To allocate the extension and location of brain tumors as well as to detect metastases, MRI examination is required. The spinal can be examined by sagittal or axial T1 and T2-weighted images, if tumor is suspected contrast-enhanced T1-weighted images are made. MRI is increasingly used in the detection of breast cancers, especially in the case of young women. Coronal T2-weighted images are typical for screening liver tumors or metastases. A study was published in the late 1990s, which demonstrated the benefits of MRI in the diagnosis of prostate cancer, since in T2-weighted images tumors can be identified as dark spots. Mostly axial images are used to examine the female pelvis [15].

B. Monitoring tumors in animal experiments

The measurement of volume of subcutaneous xenograft tumors is necessary to monitor the procession of disease as well as the efficiency of a given therapy. Non-invasive imaging techniques afford more precise and accurate results than conventionally used caliper. Gregory D. Ayers et al. [19] set ultrasound imaging against manual caliper. They have found ultrasound imaging more appropriate than MRI or CT, because of the short acquisition time, which can be relevant when numerous animals must be measured. Using manual caliper, measurement of the tumor volume is approximated with an ellipsoid in a three-dimensional coordinate system. The size of tumor can be measured along x and y axis (width and length), but the spatial extent of tumor along the third dimension, i.e. the depth can not be determined. Hence, the third dimension of the tumor is estimated with the shorter measured dimension that is defined as y in the formula. Thus, the volume of the tumor is calculated by solving the following equation for V:

$$V = \frac{xy^2}{2}, \tag{1}$$

where the tumor volume is V, x the width and y is the length of tumor.

The study of G. D. Ayers et al. [19] showed that in case of irregular tumor structure, the commonly used caliper may result in significantly different value of the tumor than the real one.

Dan Yang et al. [20] have studied gene expression in mice tumors with 4.7 Tesla small-animal MRI. Fast spin echo sequence was applied and T2-weighted images were created (TR = 4120 ms, TE = 72 ms), with 1 mm slice thickness, 0.3 mm gap setting and 256x256 image resolution. The tumor volumes were calculated from the tumor area in images multiplied by the section thickness plus intersection gap. The study suggests also MRI examination during therapy, because anatomical images can help oncology to monitor internal processes of tumor, e.g. tumor necrosis.

Peggy A. Barnett et al. [21] have determined three-dimensional tumor volume in the brain of rats infected by lung carcinoma. C. Patrick Reynolds and colleagues [22] have studied cancerous mice using MRI and X-rays.

Marzola Pasquini et al. [23] have detected antiangiogenic effect of SU11248, a novel selective multitargeted tyrosine kinase inhibitor, in vivo using MRI. M. A. Rosen and M. D. Schnall [24] have studied the vascularization of tumors, instead of tumor morphology. R. T. Ullrich and colleagues [25] presented tumor microvascularization in mice and the response to antiangiogenic therapy by 7 Tesla small-animal MRI.

Based on the above, it is clear that small-animal magnetic resonance imaging is a widespread technology to investigate mice tumors and vascularization.

IV. INVESTIGATING SUBCUTANEOUS MICE TUMORS WITH SMALL-ANIMAL MRI

A. Experimental settings

In animal researches the MRI scanners have typically higher field strength than MRI used in human studies. We used a 9.4 Tesla field strength Varian small-animal MRI to imaging subcutaneous mouse tumors. Since the MRI device makes a lot of noise during the measurement, in most cases the animals are acclimated to noise. The animals must be stationary during measurement; therefore isofluorane is applied for inhalational anesthesia. The preparation of examination continued with the intubation. Catheter was placed in the tail vein for injection - according to the mouse tail vein injection protocol [26] - to investigate drug effect.

During the measurement the life parameters of animal must be monitored. The monitoring of breathing requires piezoelectric transducer that allows scientists to calculate the respiratory rate from the detected respiratory movements of animal. The temperature of the body is measured by rectal thermometer. The decrease of the temperature of the body in anesthesia is compensated by blowing warm air. ECG recording is not impossible, but it is a serious measurement challenge. To minimize the movement of the animal, its position must be fixed. The usage of special receiver coils can improve the quality of images. The available spatial resolution depends on the acquisition time. In the case of 0.1x0.1x0.3 mm voxel size and 4-6 slices the acquisition time is about 5 minutes, however, measuring 0.015x0.015x0.3 mm voxel size and 20 slices can take 1 day. The greatest limit of acquisition time is the anesthesia. The reasonable choice is from 5 minutes to about 1 hour. Shorter acquisition time results very low image quality, longer one causes danger to the life of animal.

B. Measurements for tumor detection

Mice were injected subcutaneous with C38 colon adenocarcinoma. Tumors located in the last third of back, so the effect of respiratory movement is minimized. After the injection of tumor cells, the skin is usually fixed with metal clips, which must be removed before imaging because of the powerful magnetic field. We measured mice that were about 20 grams and had large tumors at the end of the experiment. The general preparation procedure, which is described above, was followed. Anesthesia occurred with isoflurane, the mice were intubated and fixed before measurements. Our preliminary experiment with a few number of mice with lethal sized tumor showed that also these critical state mice endured the anesthesia well. Therefore using the same anesthesia process for mice with small and large sized tumor is not risky. The breathing of mice was continuously monitored. We analyzed the results to determine the optimal setting parameters. Applying MRI to examine tumors helps to get more information about tumor growth in further animal researches.

C. Experimental results

The mice endured the anesthesia well, so the examination of mice with smaller tumor in the same way is not risky. The produces images were converted to NIfTI (Neuroimaging Informatics Technology Initiative) format, which is suitable for image processing. A NIfTI file contains all data of a measurement. It is a complex structure, which consists of header and image data. The header stores information related to the measurement, while the image is saved as a multi-dimensional matrix. An anatomical image is represented as a three-dimensional structure: *x* axis is the horizontal, *y* axis is the vertical component of image and *z* is the sequence of slices. The perpendicular plane to the field of the magnet is called axial orientation. Depending on the direction of phase encoding the measurements can be grouped into axial and axial 90°. All of our images are axial 90°. The number of slices in each case is 20 with slice thickness 2mm. TR (repetition time) is the delay between excitation of the same slice; and TE (echo time) is the time that elapses between the excitation and the detection.

T1-weighted images have been acquired using gradient-echo, spin echo and fast spin echo sequences. *Gradient-echo* sequence is not appropriate to sharply separate tumor from the surrounding tissues, because both the connective tissue and the tumor can be visualized by similar bright pixels. To improve image quality and differentiate between these tissues one must increase the average number of slices, which results in longer data acquisition time. Too high TR value produces hollow images, because TR has a large effect on the contrast and also acquisition time. Too high TE value causes in the image shadow artifacts over and under the mouse. It comes from the respiratory movements of the animal during scanning. Improving the resolution by halving the voxel size in the *x-y* plane, the inner structure of the tumor can be examined. Because of the smaller voxel size, smaller

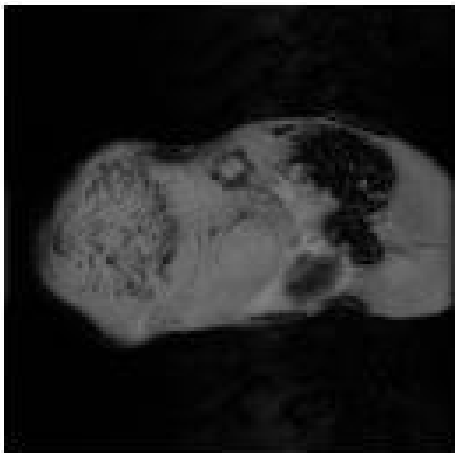

Figure 1. Gradient-echo MRI image of subcutaneous tumor in mouse (Spatial resolution: 128x128x20, voxel size: 0.3125x0.3125x2 mm, TR=500 ms, TE=2.85 ms, flip angle: 90°, averages: 4, scan time: 4 m 17 s)

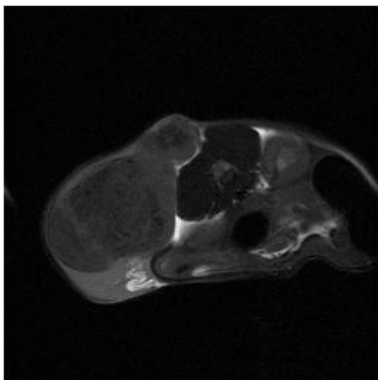

Figure 2. Fast spin echo MRI image of subcutaneous tumor in mouse
(Spatial resolution: 256x256x20, voxel size: 0.1563x0.1563x2 mm, TR=1350 ms, TE=31.47 ms, flip angle: 90°, averages: 4, scan time: 2 m 56 s)

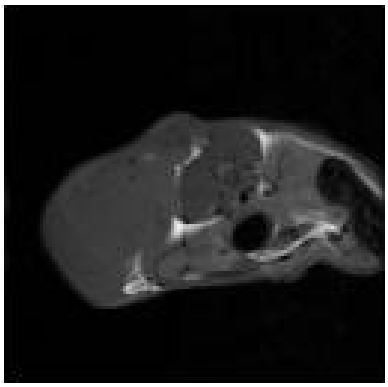

Figure 3. Spin echo MRI image of subcutaneous tumor in mouse
(Spatial resolution: 128x128x20, voxel size: 0.3125x0.3125x2 mm, TR=1000 ms, TE=10.79 ms, flip angle: 90°, averages: 4, scan time: 8 m 32 s)

volume is kindled and signals are gathered from this smaller volume, thus the volume-specific peculiarities come out.

Spin echo sequence raises the difference between tumor and surrounding tissues. Using weak parameter sets spatial resolution: 64x64x20, voxel size: 0.625x0.1625x2 mm, TR=500 ms, TE=9.42 ms, averages 1, scan time: 32 s) and very short data acquisition time, the tumor can be acceptably segmented. As we have increased acquisition time, the image became more detailed and informative. The spin echo is a commonly used sequence, because it can produce extremely good contrast, but the acquisition time strongly limits it. The *fast spin echo* (FSE) makes the data acquisition more effective, hence in addition to shorter acquisition time, high image quality and great contrast can be achieved. However, using FSE very strong gradient is needed.

Contrast agents improve the visibility, but it is an extra strain to the organism, which can be lethal to animals, which are in the final stage of cancer. We have found that measurements without contrast agents resulted in high quality images, where tumor can be circumscribed precisely, thus the usage of contrast is unnecessary. To process images homogeneous inner structure of tumor is required and distinct contours are necessary.

V. Conclusion

Determination of tumor volume is one of the most fundamental steps of modeling tumor growth and model validation. Although tumors can be approximated with ellipsoid, the conventional manual caliper measurements can result significant error. Magnetic resonance imaging provides the opportunity to trace objectively the development of the tumor. Our results showed that high-contrast anatomical images allow differentiation between tumor and surrounding tissues. With this in vivo imaging technique, tumors and their internal structure can be visualized. The fast spin echo sequence produces in short data acquisition time detailed images, which can be processed by computer. From the known parameter and segmented area, tumor volume can be calculated. Further work will contain other mouse experiments with small-animal MRI, where we will investigate tumor growth under antiangiogenic therapy. Moreover, other optimal image processing aspects will be investigated similar to [27].

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