INTRODUCTION

Development of tumors is determined by many factors including interactions between cancer cells and their environment (1,2). These factors play a role in different stages of tumor development, and inhibition of these processes to hamper tumor growth may have its effects in specific stages only (3). For a proper understanding of tumor development and the effects of intervention studies, longitudinal analysis of tumor development would be of great help (4).

Subcutaneously implanted tumors can be analyzed rather easily in a quantitative manner in time using calipers (5,6), but these tumors do not develop in their natural environment. To mimic tumor development and metastasis in humans as closely as possible, orthotopic tumor growth is preferred (7–9). A number of noninvasive imaging modalities are available to longitudinally monitor tumor development in small animals such as bioluminescence imaging (BLI), fluorescence imaging (FLI), magnetic resonance imaging (MRI), computed tomography (CT), ultrasonography (US), single photon emission CT (SPECT), and positron emission tomography (PET) (4,10–12).

BLI and FLI are the methods of choice to quantitate tumor load in animals, because the methods are reasonably valid when imaging is performed in a standardized manner (13,14). They are rapid, relatively inexpensive, and easy to perform. The spatial resolution of BLI is low, whereas FLI has higher spatial resolution than BLI (14–16). Cancer cells have to be genetically modified for BLI and FLI, because the genes encoding for luciferase, green fluorescent protein (GFP), or related proteins have to be transfected. This may affect the immunogenicity of cancer cells (13). Furthermore, the luminescence and fluorescence signal is quenched by tissues and particularly hair on skin.

Noninvasive magnetic resonance imaging of the development of individual colon cancer tumors in rat liver

Olaf R.F. Mook¹, Ard Jonker¹, Aart C. Strang¹, Andor Veltien², Giulio Gambarota², Wilma M. Frederiks¹, Arend Heerschap², and Cornelis J.F. Van Noorden¹

Monitoring tumor development is essential for the understanding of mechanisms involved in tumor progression and to determine efficacy of therapy. One of the evolving approaches is longitudinal noninvasive magnetic resonance imaging (MRI) of tumors in experimental models. We applied high-resolution MRI at 7 Tesla to study the development of colon cancer tumors in rat liver. MRI acquisition was triggered to the respiratory cycle to minimize motion artifacts. A special radio frequency (RF) coil was designed to acquire detailed T1-weighted and T2-weighted images of the liver. T2-weighted images identified hyperintense lesions representing tumors with a minimum diameter of 2 mm, enabling the determination of growth rates and morphological aspects of individual tumors. It is concluded that high-resolution MRI using a dedicated RF coil and triggering to the respiratory cycle is an excellent tool for quantitative and morphological analysis of individual diffusely distributed tumors throughout the liver. However, at present, MRI requires expensive equipment and expertise and is a time-consuming methodology. Therefore, it should preferably be used for dedicated applications rather than for high-throughput assessment of total tumor load in animals.

Figure 1. (A) T1-weighted and (B) T2-weighted magnetic resonance (MR) images from the same 2-mm-thick optical section of a rat liver at 5 weeks after induction of colon cancer tumors. Three tumors are faintly visible as hyperintense structures in the T1-weighted image (white arrows), whereas all tumors are clearly visible as hyperintense lesions in the T2-weighted images (encircled in red). Scale bar, 1 cm.

¹University of Amsterdam, Amsterdam and ²University Medical Center St. Radboud, Nijmegen, The Netherlands
Nude or shaven mice are used for BLI and FLI to minimize quenching (13,17). Light has to traverse tissues only once for BLI (light is generated by the luciferase enzyme) and twice for FLI (both excitation and emission light).

MRI has shown its merits for morphological imaging, because it has a high spatial resolution especially in soft tissues, it does not require transfection of cancer cells or radioactivity, and hair on the skin does not interfere with the signal. However, animal MR systems are expensive, expertise is required, and the slow imaging procedures do not allow high-throughput screening (10–12); however, specific approaches are emerging to improve this situation (18). Therefore, MRI has to be used where other imaging techniques have failed.

MRI has proven its value for imaging solitary tumors such as intracranial glioma (19–21) and metastases (22), orthotopic prostate (23) and breast tumors (24), and ectopic subcutaneous tumors (25,26). These tumors can be imaged with the use of long image capturing times because motion artifacts in the living animals do not play a significant role in these locations. MRI has also been applied to quantify tumor load in organs that are subject to movement due to heart beat and/or breathing. These organs include lung (27), colon (28), pancreas (29,30), stomach (31), and liver (32–36).

In the present study, MRI is applied to study quantitatively and morphologically the growth and development of individual diffusely distributed colon cancer tumors in rat liver. An optimized technique was used to perform T1- and T2-weighted MRI at 7 Tesla with a dedicated radiofrequency (RF) microstrip coil to increase the signal-to-noise ratio in combination with respiratory triggering to minimize motion artifacts (37).

MATERIALS AND METHODS

Animals

Colon cancer tumors were induced as described previously (38) in adult male Wag-Rij rats (n = 26; Broekman, Someren, The Netherlands) with a body weight of 200–250 g after being maintained for 2 weeks under constant environmental conditions with free access to food and water. All animal experiments were approved by the Animal Care and Use Committees of the Academic Medical Center at the University of Amsterdam and the Nijmegen University Medical Center.

Colon Cancer Cells

An established colon carcinoma cell line, CC531s, was used. The cells were cultured at 37°C as monolayers in RPMI-1640 Dutch modification without L-glutamine (Gibco/BRL, Grand Island, NY, USA) supplemented with 10% (v/v) fetal calf serum, 2 mM L-glutamine, 100 IU penicillin/mL, and 100 mg streptomycin/mL (all from Gibco/BRL). Cells were washed with phosphate-buffered saline (PBS) and detached with trypsin (0.05% w/v; Gibco/BRL) and EDTA (0.02% w/v; Sigma, St. Louis, MO, USA) in PBS. After centrifugation at 250×g at room temperature for 5 min, cell suspensions were obtained with a viability of at least 95%.

Surgery

A small midline incision was made in the abdominal wall of rats under anesthesia with fentanyl-fluanizone-midazolam mixture (FFM; 1 mL Hypnorm, 1 mL midazolam, and 2 mL water, 0.27 mL/100 g body weight, intraperitoneally). A suspension containing 5 × 10⁶ cancer cells in 500 μL PBS was injected into the portal vein with a 27-gauge needle.

Magnetic Resonance Imaging

MRI of the livers of each rat was performed at various time points during 5 weeks after administration of the cancer cells. A maximum of six animals per day could be imaged. The animals were anesthetized with 1.5% isoflurane...
and a mixture of N₂O/O₂ (1:1). Body temperature was maintained at 37°C by covering the rats with a bed heated with circulating warm water. A dedicated 5 × 7 cm microstrip RF coil of novel design was used as a transmitter/receiver to improve the quality of the images (37). A customized plastic cradle was built to accommodate the rat in the prone position on the coil.

MR data were collected on a SMIS MR console (Surrey Medical Imaging Systems, Surrey, UK) interfaced with a 7 T/200-mm horizontal bore magnet (Magnex Scientific, Abingdon, UK) and a 150 milli-Telsa/meter (mT/m) gradient insert. After initial monitoring of the liver with fast gradient-echo scout images, 16 contiguous coronal images (i.e., parallel to the coil) were acquired. The acquisition protocol consisted of T1- and T2-weighted spin echo images for anatomical localization of the liver and detection of tumors (37). Imaging parameters were: image matrix size of 512 × 512, field of view of 14 × 14 cm, slice thickness of 2 mm, and one signal average per phase-encoding step. The values of repetition time (TR) and echo time (TE) were TR/TE = 500/15 ms for the T1-weighted images and TR/TE = 710/36 ms for the T2-weighted images. Image acquisition was triggered to the respiration rate monitored by an optical probe (Siracust 401; Siemens, Erlangen, Germany) as described in detail in Gambarota et al. (37).

Data Analysis

Individual tumors were selected in a specific T2-weighted dataset. Then, the same tumors were identified in T2-weighted datasets taken at other time points. Individual tumor mass was calculated by two independent methods that gave comparable results. One method was based on the sum of all tumor surface areas (mm²) times slice thickness (mm). The second method was based on the image slice with the largest tumor surface area for the calculation of tumor mass.

Figure 4. T2-weighted magnetic resonance (MR) images from two adjacent 2-mm-thick optical sections (A, B, C, D and E, F, G, H) of a rat liver. The images were taken at (A and E) day 11, (B and E) day 18, (C and F) day 21, and (D and H) day 26 after inoculation of colon cancer cells. A tumor becomes visible at day 18 (encircled in red) and has disappeared on day 21. Scale bar, 1 cm.
As all tumors were more or less globular, the radius was calculated from the largest surface area using $r = \sqrt{\text{surface area}/\pi}$. The volume was calculated as $v = \frac{4}{3} \pi r^3$. Tumor volume doubling times were calculated on the basis of best fitting exponential curves representing the relationship between volume of individual tumors and time.

RESULTS

The use of a breathing-triggered MRI protocol enabled sharp and detailed T1-weighted images of the liver and delineation of intrahepatic structures (Figure 1A). T1-weighted imaging did not allow clear visualization of tumors in the liver; some but not all tumors were detected as slightly hyperintense structures (Figure 1A). However, tumors could be clearly identified as hyperintense lesions in T2-weighted images (Figure 1B). A minimum diameter of 2 mm was required for visualization due to voxel size ($0.27 \times 0.27 \times 2.0$ mm). It took approximately 2 weeks before the first tumors could be visualized with MRI. This limitation in spatial resolution is mainly due to the short periods available for imaging during the respiratory cycle of the animals. Fat tissue was also hyperintensive and even more so than tumors in both T1- and T2-weighted images (Figure 1). It was discriminated from tumors on the basis of morphology and the fact that it is not present within the liver.

Colon cancer tumors developed as solitary globular tumors even when the liver was full of tumors (Figure 2), but sometimes collision of tumors was observed (Figure 3). Only few tumors disappeared in the period of imaging (3–5 weeks after cancer cell inoculation) (Figure 4).

Imaging of the livers at three or more time points enabled us to follow quantitatively tumor development in time (Figure 5). Exponential fitting of the volumes of individual tumors enabled the calculation of tumor volume doubling time as a measure of tumor growth rate. The average tumor volume doubling time was $0.6 \pm 0.1$ week or approximately 4 days in 25 individual tumors in 12 rats. It appeared that the volume doubling time of all individual tumors was similar, whereas their volume varied manifold. This indicated that the starting point of tumor growth varied in time (Figure 6), but once the tumors grew, growth rates were similar. Unfortunately, the MRI approach that we used did not allow visualization of tumors in the first 2 weeks after cancer cell inoculation to establish what the fate was of the $5 \times 10^5$ inoculated cancer cells that ultimately resulted in only 0–100 tumors per liver (38) or to show different onsets of tumor development.

DISCUSSION

Studies in the early 1990s have shown that MRI is a powerful tool to quantify tumor load in live animals (32,34) and the effects of therapy on tumor development (19,33). However, MRI of small animals, requiring specialized expensive equipment, technological expertise, and imaging procedures, is slow. In our study, MRI of six animals was the maximum that could be achieved during a working day. Therefore, MRI is not yet the imaging methodology of choice for large-scale determination of tumor load in animals and the effects of therapy thereupon. For these purposes, a simple, rapid, and relatively cheap imaging methodology such as BLI (13) and FLI (6,14–16) is required. In this context, MRI may be more appropriate for the analysis of the development of individual tumors in animals, both morphologically and quantitatively, as shown in the present study. A combination of MRI and BLI (39–42) or MRI and FLI (43,44) provides complementary information.

MRI of tumors does not rely on transfection of cancer cells with any reporter gene, unlike BLI and FLI. Imaging can be performed noninvasively multiple times, allowing the tracking of tumor development over time. However, MRI is limited by the spatial resolution and the need for specialized equipment and expertise. Future developments in MRI technology may improve its applicability for large-scale studies of tumor development in live animals.
times, and tumors can be imaged in any part of the animal. Therefore, MRI provides a valuable tool for imaging tumor development in live animals. MRI technology has improved considerably during the last decade by the implementation of stronger magnetic fields (23,28,30,37,45), modified coils, and the improvement of imaging protocols that has resulted in better signal-to-noise ratios and increased spatial resolution (37,46,47) and higher throughout approaches (18).

The routine procedure used in the present study enabled imaging of tumors with a minimum diameter of 2 mm. MRI of the liver in live animals is difficult due to motion artifacts caused by respiration. Triggering of the MRI protocol to the breathing of the animal enabled us to acquire well-contrasted and detailed images of rat livers and to image tumor development of colon cancer in the liver, as was also shown by Garbow et al. (27) and Cai et al. (36). These improvements in MRI technology enable good quality noninvasive imaging of tumors in living animals.

Quantification of growth of individual tumors appeared to be possible. Tumor volume doubling times obtained by exponential curve fitting of tumor volumes at 3, 4, and 5 weeks showed that the volume doubling time was similar in all tumors. It suggests that tumors have different onsets in growth rather than different growth rates to explain differences in sizes at the time of sacrifice of the animals.

A number of technological modifications of MRI may be of interest to further improve contrast and thus spatial resolution, such as the use of the contrast agents gadolinium (21,24,28,29) and manganese (48) in T1 imaging or iron oxide particles (25) in T2 imaging. Other MRI approaches such as diffusion MRI (20,49), which is sensitive to cellular density, and organization or dynamic contrast-enhanced MRI (26,50,51), which reflects vascular functionality, are powerful approaches for the functional characterization of individual tumors. Recently, MRI of targeted agents is showing great promise (52,53).

Finally, Heyn et al. (54) reported an MRI study at the single-cell level of breast cancer metastasis in mouse brain. Cancer cells were labeled with micron-sized iron oxide particles. This methodology allows the study of early events in the development of metastases to address questions, such as what is the fate of cancer cells when entering the circulation and/or the organ where secondary growth takes place, the onset of growth, or the elimination of a tumor.

In conclusion, this study shows that MRI is a highly sophisticated technique that can be applied to address specific research questions in tumor biology, such as the analysis of the development of individual tumors in time both morphologically and quantitatively.


Received 17 September 2007; accepted 7 November 2007.