The Utility of Micro-CT and MRI in the Assessment of Longitudinal Growth of Liver Metastases in a Preclinical Model of Colon Carcinoma

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Rationale and Objectives: Liver is a common site for distal metastases in colon and rectal cancer. Numerous clinical studies have analyzed the relative merits of different imaging modalities for detection of liver metastases. Several exciting new therapies are being investigated in preclinical models. But, technical challenges in preclinical imaging make it difficult to translate conclusions from clinical studies to the preclinical environment. This study addresses the technical challenges of preclinical magnetic resonance imaging (MRI) and micro-computed tomography (CT) to enable comparison of state-of-the-art methods for following metastatic liver disease.

Materials and Methods: We optimized two promising preclinical protocols to enable a parallel longitudinal study tracking metastatic human colon carcinoma growth in a mouse model: T_2-weighted MRI using two-shot PROPELLER (Periodically Rotated Overlapping ParallEL Lines with Enhanced Reconstruction) and contrast-enhanced micro-CT using a liposomal contrast agent. Both methods were tailored for high throughput with attention to animal support and anesthesia to limit biological stress.

Results and Conclusions: Each modality has its strengths. Micro-CT permitted more rapid acquisition (<10 minutes) with the highest spatial resolution (88-micron isotropic resolution). But detection of metastatic lesions requires the use of a blood pool contrast agent, which could introduce a confound in the evaluation of new therapies. MRI was slower (30 minutes) and had lower anisotropic spatial resolution. But MRI eliminates the need for a contrast agent and the contrast-to-noise between tumor and normal parenchyma was higher, making earlier detection of small lesions possible. Both methods supported a relatively high-throughput, longitudinal study of the development of metastatic lesions.

Key Words: PROPELLER; MR; CT; mice; liver metastases.

Colorectal cancer is the third most common type of cancer in humans (1). It commonly metastasizes to the liver, at which point morbidity and mortality drastically increase. In a third of the patients who die of colorectal cancer, metastatic disease is found only in the liver. Liver metastases are also seen in other cancers such as pancreas, stomach, breast, and lung, making the liver one of the most common sites of distal metastases, second only to lymph nodes. Early detection and effective treatment of liver metastases would greatly improve prognosis for many patients.

Preclinical orthotopic disease models, which closely mimic human tumor conditions, are a tremendous resource for measuring the efficacy of many potential treatments now under study. Preclinical imaging is rapidly becoming one of the most critical methods for evaluating response to these therapies. But, extension of clinical methods/conclusions to the preclinical environment is fraught with challenges. The mouse, at 25 g, is nearly 3000 times smaller than a human, so the spatial resolution in the preclinical system must be commensurately higher. Physiologic rates are also faster (heart rate is 10 times and respiration is 5 times faster), so the temporal resolution of the preclinical system must be correspondingly faster. Small animal imaging usually requires anesthesia, and respiratory motion is a major technical challenge, particularly for imaging abdominal organs. Although scan-synchronized respiration has become routine, it requires intubation, which

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induces stress in the animal and adds to the complexity of the study. The mouse is fragile. One must provide external thermal regulation and limit physical handling. Finally, for any protocol to be useful, it must be executed in a reasonable time. Thus the criteria we have set in designing this comparative preclinical protocol are: 1) stress on the animal must be minimized; 2) setup and execution must be accomplished in <30 minutes; 3) imaging must cover the entire liver; and 4) images must be of the highest quality possible.

This last criterion of image quality imposes a particularly vexing conundrum. What are the most appropriate preclinical modalities and how does one optimize them for our given task—noninvasive study of liver metastases? Preclinical studies of mouse models of liver cancer have used several imaging modalities: positron emission tomography (PET) (2), bioluminescence imaging (3,4), computed tomography (CT) (5), and magnetic resonance imaging (MRI) (6–8), albeit independently. PET provides excellent functional information regarding tumor metabolism. However, PET is costly, not widely available, and has resolution limits of >1 mm^3 imposed by the physics of positron decay. Although the spatial resolution limit is not a significant problem in the clinical domain, resolution at 1 mm or greater is particularly problematic in the mouse. Bioluminescence imaging, though highly sensitive, is also limited by spatial resolution as well as the need for mouse models that genetically express luciferase. CT is more readily available, provides high spatial resolution, and is also preferred for clinical liver imaging, which makes translational studies more appealing. MRI has the best soft-tissue contrast and has been used frequently with an extraordinary range of imaging sequences and contrast mechanisms. Thus, we chose to compare micro-CT and MRI microscopy.

We needed to determine how to optimize these two modalities. Clinical systems have for the most part been standardized. But, preclinical systems vary widely. A micro-CT system with a 10W x-ray tube requires considerably longer scan time than a 50-kW tube CT system, for constant noise. The contrast and noise in an MR image is dependent on a host of technical choices, such as field strength, imaging strategy (e.g., T_1-weighted, T_2-weighted, diffusion-weighted), use of contrast agents, and so on. Because of the much wider variation in technical specifications and approaches for preclinical imaging, we have optimized two imaging strategies that we believe represent the state-of-the-art for preclinical micro-CT and MRI microscopy.

The three determinants of image quality in micro-CT are resolution, signal-to-noise ratio (dose), and contrast. We previously described a micro-CT system that supports 88-μm isotropic spatial resolution with scan time <5 minutes. This system has a unique design that allows us to use multiple, high-power, rotating anode x-ray tubes that deliver radiation flux >250 times that of commercial systems using microfocal tubes (9). Thus, the quantum noise is very low, resolution is high, and scan times are short. The Achilles heel of CT is contrast difference between metastatic and normal tissue. Conventional contrast agents are problematic in preclinical models, because they generally clear faster than the time required for completing the scans. We have addressed this by using a liposome contrast agent, which unlike conventional renal clearance, has a slower clearance via the hepatic system.

Optimizing resolution and contrast in preclinical MRI is difficult, again because of the unique challenges of preclinical imaging. The highest-resolution preclinical MRI systems operate at fields >7T. At these fields, T_1 values for all tissues are considerably longer than at clinical fields (≤3T), and T_2 values are considerably shorter. T_2-weighted imaging, one of the most common methods for detecting liver metastases, requires a different approach for a preclinical system. We have previously demonstrated a technique for preclinical T_2-weighted imaging at high-fields using fast spin echo–based multishot PROPELLER (Periodically Rotated Overlapping ParallEL Lines with Enhanced Reconstruction) (10,11). The motion correction ability of PROPELLER acquisition and reconstruction (12) makes this technique ideal for imaging free-breathing mice. The fast spin echo–based approach enables excellent T_2-weighted contrast. These characteristics led us to choose this T_2-weighted sequence as representative of state-of-the-art technology in preclinical MRI of metastatic lesions. The sequence has been tuned to allow acquisition of free-breathing animals in 30 minutes. The goal of this study was to compare these two optimized (micro-CT and MR) imaging strategies to detect liver metastases in a mouse model. To the best of our knowledge, a rigorous preclinical study like this has not been previously undertaken.

MATERIALS AND METHODS

Animal Model

All animal studies were approved by the Duke University Institutional Animal Care and Use Committee. Tumor inoculation procedures were performed at Piedmont Research Center (Morrisville, NC). Female athymic nude mice were implanted with 5×10^6 cells of HT29 colon carcinoma in 50 μL volume of using a 25-gauge needle in the spleen. After a 2-minute pause post-injection, a splenectomy was conducted under isoflurane anesthesia. Animals were allowed to recover for 9 days before imaging.

This model was unique because it enabled study of secondary tumor sites. The primary tumor site, which normally dominates the disease model, was removed by splenectomy, which allowed sufficient time for development of secondary tumor sites—in this case, liver metastases. For colon carcinoma, the liver is the primary site of metastases. This was also a very aggressive disease model. After 2 weeks, the liver tumors showed exponential growth. Thus, the total study duration was restricted to approximately 1 month after implantation, at which point the mice had reached the humane end point for acceptable tumor burden.