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Detection of *in situ* mammary cancer in a transgenic mouse model: *in vitro* and *in vivo* MRI studies demonstrate histopathologic correlation

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Abstract

Improving the prevention and detection of preinvasive ductal carcinoma *in situ* (DCIS) is expected to lower both morbidity and mortality from breast cancer. Transgenic mouse models can be used as a ‘test bed’ to develop new imaging methods and to evaluate the efficacy of candidate preventive therapies. We hypothesized that despite its microscopic size, early murine mammary cancer, including DCIS, might be accurately detected by MRI. C3(1) SV40 TA_g female mice ($n = 23$) between 10 and 18 weeks of age were selected for study. Eleven mice were subjected to *in vitro* imaging using a T₂-weighted spin echo sequence and 12 mice were selected for *in vivo* imaging using a T₁-weighted gradient echo, a T₂-weighted spin echo and high spectral and spatial resolution imaging sequences. The imaged glands were carefully dissected, formalin fixed and paraffin embedded, and then H&E stained sections were obtained. The ratio of image-detected versus histologically detected cancers was obtained by reviewing the MR images and H&E sections independently and using histology as the gold standard. MR images were able to detect 12/12 intramammary lymph nodes, 1/1 relatively large (~5 mm) tumor, 17/18 small (~1 mm) tumors and 13/16 ducts distended with DCIS greater than 300 μm . Significantly, there were no false positives—i.e., image detection always corresponded to a histologically detectable cancer in this model. These results indicate that MR imaging can reliably detect both preinvasive *in situ* and early invasive mammary cancers in mice with high sensitivity. This technology is an important step toward the more effective use of non-invasive imaging in pre-clinical studies of breast cancer prevention, detection and treatment.

Introduction

Women diagnosed with breast cancer today have significantly better survival outcomes compared with their counterparts 30 years ago (Jemal *et al* 2004). This is attributed to improvements in treatment as well as improved detection of early stage cancer due to screening mammography (Berry *et al* 2005). Currently, 15–25% of newly diagnosed breast cancers are preinvasive ductal carcinoma *in situ* (DCIS) (Tsikitis and Chung 2006), and with improvements in imaging these percentages are likely to increase. Women diagnosed with DCIS have the best prognosis with long-term survival rates of 97–99% (Morrow *et al* 2002). Half of all newly diagnosed invasive carcinomas are stage I, which is the earliest form of invasive breast cancer and does not involve metastatic spread to the lymph nodes (Li *et al* 2003). Some have suggested that improving the detection of early cancers is essential for further decreasing mortality rates (Duffy *et al* 2003). Thus, to help increase survival rates it is clearly essential to improve detection and effective treatment of early breast cancer.

Dynamic contrast-enhanced MR imaging (DCEMRI) of the breast has been shown to improve the detection of early stage invasive cancers, and has recently been recommended by the American Cancer Society for the screening of women at high risk for developing breast cancer (Saslow *et al* 2007). However, initial reports studying the presentation of DCIS on DCEMRI found poorer sensitivity and specificity compared with x-ray mammography (Gilles *et al* 1995, Menell *et al* 2005, Orel *et al* 1997, Schouten van der Velden *et al* 2006). Although recent work has demonstrated that the sensitivity of DCEMRI for DCIS is increasing (Kuhl *et al* 2007), there is clearly room for improvement in diagnostic accuracy. It is anticipated that studies of the physiological and biological characteristics of early breast cancers will help improve imaging methods and analysis, because these insights will help to guide imaging approaches to find physiologically abnormal tissues seen in cancer.

Due to the urgency of surgery in early human breast cancer, studies of the natural history of such cancers in patients are difficult to perform. Therefore, transgenic and xenografted mouse models of breast cancer are widely used to investigate the biological basis of human breast cancer, to evaluate new therapies and to develop improved imaging methods. The usefulness of these mouse models depends on how closely they resemble human breast cancer. This is one reason why transgenic mouse models are appealing and have led to improvements in detection and treatment of cancers: the tumors arise without additional carcinogens and the early tumors progress through the stages of disease, i.e. from *in situ* to invasive, closely mimicking their human counterpart. If mice are to be used as successful models of human cancer biology, then imaging methods that detect *in situ* tumors are required to accurately assess preventive, diagnostic and therapeutic interventions. To date, however, there have been no reports of *in vivo* imaging of *in situ* or even nonpalpable invasive mammary gland cancers in mice (Abbey *et al* 2004, Artemov *et al* 2003, Bremer *et al* 2005, Galie *et al* 2004, Geninatti Crich *et al* 2006, Hsueh *et al* 2006, Jenkins *et al* 2005, Robinson *et al* 2003, Rodrigues *et al* 2004, 2006, Seemann *et al* 2006, Tian *et al* 2003). In fact, most imaging studies of mouse mammary cancer have focused on large tumors that are extremely advanced. Relative to DCIS and early invasive cancers, these more advanced cancers are not realistic models of the majority of newly diagnosed breast cancers in women.

In this project, our goal was to determine whether MR imaging of early murine mammary cancer, including *in situ* carcinoma, is feasible. We studied the SV40 Tag transgenic mouse model of breast cancer in which mammary cancer develops at about 16 weeks and progresses through histological stages that are similar to human breast cancer progression. We developed our imaging technique by first detecting microscopic cancers *ex vivo* in excised mammary glands. We then were able to successfully advance to *in vivo* imaging of *in situ* carcinoma in living animals.

Materials and methods

Animals

Twenty-three C3(1) SV40 large T antigen (Tag) transgenic mice were used for MR imaging (Maroulakou *et al* 1994). This mouse model targets expression of large Tag to the female mammary gland via the C3(1) promoter. Female mice develop mammary cancer that resembles human ductal breast carcinoma, including progression through atypical ductal hyperplasia (~8 weeks), DCIS (~12 weeks) and IDC (~16 weeks) (Green *et al* 2000). Eleven of the 23 mice were selected for *in vitro* imaging, and the remaining 12 for *in vivo* imaging. All procedures were carried out in accordance with our institution's Animal Care and Use Committee approval. Animals were anesthetized prior to imaging experiments, and anesthesia was maintained during imaging at 1.5% isoflurane. Body temperature was maintained with a warm air blower. The temperature, heart rate and respiration rate were monitored with data taken every minute and the signal from the respiration sensor was used to obtain gated images.

MRI experiments

Imaging was performed with a Bruker 4.7 tesla magnet equipped with a self-shielded gradient set that delivers maximum gradient strength of 20 Gauss cm⁻¹.

In vitro—A homebuilt 6-leg low-pass half-open birdcage coil (3 cm length × 2 cm width × 1 cm height) was built for mammary gland *in vitro* imaging using a multi-slice multiple spin-echo sequence (rapid acquisition with refocused echoes (RARE) (Friedburg *et al* 1987), four RARE partitions, TR/TE: 4000/50 ms, field of view (FOV) = 3.0 × 1.5 cm, number of excitations (NEX) = 2, slice thickness = 0.75 mm and inplane resolution = 117 μm). Twenty-two excised and fixed inguinal mammary gland specimens were imaged from 11 mice between 8 and 22 weeks of age. Inguinal mammary glands are *I*-shaped with a typical size of 2 cm × 2 cm and 2–3 mm thick, unless a larger tumor is present. For MR imaging, the glands were laid flat in the coil and three to seven slices were obtained from the top down.

In vivo—Another homebuilt 8-leg low-pass half-open birdcage coil (3 cm length × 3 cm width × 2 cm height) that produced high flux density in the mammary gland (Fan *et al* 2006) was used for *in vivo* imaging. Several pulse sequences were evaluated. Initially, two sets of multislice gradient-recalled echo (GRE) images were obtained (TR/TE: 675/7 ms, FOV = 3.0 × 3.0 cm, matrix size = 256 × 256, NEX = 2, slices = 21, slice thickness = 0.5 mm, in-plane resolution = 117 μm and flip angle = 30°) across the entire sensitive volume of the coil

to map out the whole gland. Based on this initial evaluation, six to ten slices that contained structures of interest (i.e., candidate cancers) were evaluated further: (i) GRE with fat suppression (same imaging parameters as above), (ii) spin-echo (SE) images (multi-slice RARE, TR/TE: 3000/29 ms, RARE acceleration factor = 4, FOV = 3.0×3.0 cm, matrix size = 256×256 , NEX = 2, slice thickness = 0.5 mm and in-plane resolution = 117), (iii) SE with fat suppression. Finally, in order to improve fat suppression, high spectral and spatial (HiSS) resolution imaging was obtained of a single slice (using echo-planar spectroscopic imaging (EPSI) (Mansfield 1984) with a spectral resolution of ~6 Hz, FOV = 3.0×3.0 cm, matrix size = 256×256 , number of echoes = 32, NEX = 2, slices = 1, slice thickness = 0.5 mm and in-plane resolution = 117 μ m). The HiSS method has been detailed in prior work (Du *et al* 2005); briefly, HiSS acquisitions sample the entire free induction decay in each voxel, and after processing water peak-height images can be displayed—this provides complete fat suppression. With a typical mouse respiration rate of less than 1 s under anesthesia (equivalent to TR ~900 ms), the time required for each set of gated GRE images was approximately 7.5 min (5.8 min without gating), and for each set of gated SE images approximately 6.5 min (6.4 min without gating). Finally, it took approximately 7.5 min to acquire one slice using HiSS (approximately 6.8 min non-gated), which is considerably less efficient than the GRE acquisition that acquired many more slices in the same amount of time. The total experiment time was approximately 1.5 h.

The inguinal mammary glands on the left side of 12 mice between the ages of 10 and 18 weeks were selected for imaging. To facilitate spatial correlations between MR images and histology (below), a fine polyethylene mesh ~3.0 cm $\times$ 2.0 cm in size with 3.0 mm spacing was embedded in partially deuterated agar and wrapped around each mouse. It also served to eliminate the air–tissue interface near the mammary gland, which is expected to reduce susceptibility artifacts.

Correlation of MRI with histology

Hematoxylin and eosin (H&E) stained sections of the imaged mammary glands were obtained (5 μ m thick H&E sections every 50 μ m) and evaluated by an experienced breast and mouse mammary gland pathologist (TK). Intramammary lymph nodes, invasive tumors and ducts distended with DCIS with diameters greater than 300 μ m were identified and used as the gold standard. For the *in vitro* study, the H&E stained sections were acquired in the same orientation as the MR images and thus the two were easily compared, after being reviewed independently. For the *in vivo* study, the agar grid served as a coordinate system of fiducial markers with which to compare the H&E stained sections of the whole gland with axial MR images, which represent cross-sectional slices through the mammary gland. During imaging, the agar grid was wrapped around the mouse and the grid positions were marked on the skin. After excision and H&E staining, the mammary glands maintained the same orientation relative to these skin markers since they remained attached to the skin throughout. We were thus able to infer the grid coordinates of cancers found on the H&E sections. In addition, the agar grid was MR-visible; the grid coordinates of candidate lesions could therefore be determined directly from the MR images. To determine the sensitivity of MRI: (i) one representative H&E section was selected per mouse and the grid coordinates of cancers were noted by an experienced pathologist, (ii) the MR images were reviewed

independently by a separate reader, and grid coordinates of candidate lesions were noted, (iii) using histology as the gold-standard for diagnosis, the locations of cancers found on the H&E section were compared with the location of lesions detected by MRI, and the ratio of MR-detected versus H&E confirmed cancers was calculated.

Image analysis

The signal-to-noise ratio (SNR) of lymph nodes, DCIS and invasive tumors was calculated in the GRE and RARE SE images as follows:

$$SNR = \frac{\bar{S}}{\sigma_{noise}},$$

where $\bar{S}$ is the average signal intensity in a region of interest (ROI) drawn around the lesion or lymph node, and σ_{noise} was averaged from the standard deviations of signal intensities measured in a 0.5 cm × 0.5 cm ROI drawn in the lower left and right corners of the image. In addition, the contrast-to-noise ratio (CNR) of lymph nodes, DCIS and invasive tumors was calculated relative to muscle and normal mammary glandular tissue (MGT) as follows:

$$CNR_{lesion-muscle} = SNR_{lesion} - SNR_{muscle}$$

$$CNR_{lesion-MGT} = SNR_{lesion} - SNR_{MGT}.$$

Lesion morphology—The morphology of the lesions and lymph nodes detected by *in vivo* noncontrast MRI was analyzed using descriptors analogous to those used for clinical contrast-enhanced breast MRI of women. For clinical examinations, the Breast Imaging-Reporting and Data System (BI-RADS) lexicon classifies the type, shape, margins and enhancement pattern of the lesion (ACR 2003). Although contrast was not used in our study, the morphology of the lesions was classified using an approach analogous to a simplified version of the BI-RADS lexicon as follows: type (mass or non-mass), shape/distribution (for mass lesions: round, oval, lobular or irregular; for non-mass lesions: linear, ductal or segmental), margins (for mass lesions only: smooth or irregular) and pattern (for mass lesions: homogeneous or heterogeneous; for nonmass lesions: homogeneous, stippled or clumped).

Results

In vitro MRI

H&E stained sections were obtained from six of the 22 excised mammary gland specimens. Analysis of the histologic slides confirmed that many stages of the development of mammary carcinoma were present in the specimens, including DCIS, small invasive tumors (<3 mm) and large tumors (>3 mm). Figure 1 shows four representative examples of the correlation between RARE SE MR images and histology. After reviewing the MR images and H&E sections separately using the pathologist's report as the gold standard for cancer

diagnosis, it can be seen that the MR images matched the H&E stained sections, demonstrating intramammary lymph nodes, DCIS and both large and small invasive tumors. Review of the MR images of all 22 excised specimens demonstrated 6 large tumors (>3 mm), 30 small tumors (<3 mm), 32 DCIS lesions and 22 lymph nodes.

In vivo MRI

An *in vivo* MR image of a normal mammary gland is shown in figures 2(a) and (b), demonstrating that after fat suppression the signal from the background mammary gland decreases. However, careful inspection reveals a diffuse background signal that may be due to normal parenchyma. Figures 2(c) and (d) also demonstrate the procedure used in this study to correlate MR images with histology using the agar grid. We found that DCIS and early invasive tumors appeared clearly against a darker background of mammary glandular tissue/fat. DCIS lesions were typically a few millimeters in length and 0.5 mm wide, while invasive tumors were small and round. Two representative examples illustrating the correlation between axial GRE MR images and histology are shown in figure 3. The MR images correlated well with the corresponding H&E stained sections of the mammary glands. H&E stained sections were obtained from the inguinal glands of all of the 12 mice selected for *in vivo* MR imaging. Based on the histologic review of the pathologist, there were 12 lymph nodes, one large (~5 mm) tumor, 18 small nonpalpable tumors ~0.5–3 mm in size and 16 ducts distended with DCIS greater than 300 μ m in diameter. The sensitivity of GRE imaging was 100% for lymph nodes (12/12); 100% for tumors larger than 5 mm (1/1); 94% for small tumors 0.5–3 mm in size (17/18); and 81% for DCIS (13/16). Significantly, there were no false positives—i.e., an MR finding corresponded to cancer in all glands examined. Three more examples of early murine mammary cancer are shown in figure 4.

The GRE images with fat suppression provided the clearest images of early murine mammary cancer. In comparison, T₂-weighted RARE images with and without fat suppression did not depict the cancers or lymph nodes well, as shown for one case in figure 5. This qualitative observation was validated by calculations of SNR and CNR, as shown in table 1. For GRE images with fat suppression, the average SNR of lymph nodes, tumors and DCIS lesions were comparable to each other and to muscle, but were three to four times higher than normal mammary gland tissue. The average SNR of lymph nodes, tumors and DCIS lesions in RARE SE images with fat suppression were higher than muscle. However, unlike GRE images, RARE SE images of early murine mammary cancers and lymph nodes had comparable SNR to the normal mammary gland tissue. Thus, because of the high background signal of the mammary gland tissue, early cancer was not well-visualized on RARE SE images. In contrast, HiSS water peak-height images provided excellent lesion visualization with complete fat suppression (figure 5).

Lesion morphology—The morphology of tumors, DCIS and lymph nodes was assessed on GRE images with fat suppression. These images were acquired on a subset of slices and contained a total of 11 lymph nodes, 9 invasive tumors and 12 DCIS lesions. Nine of 9 invasive tumors were mass lesions, with a round (6/9) or irregular (3/9) shape, with smooth (6/9) or irregular (3/9) margins, and with a homogeneous (7/9) pattern. As with invasive tumors, 11/11 of lymph nodes were mass lesions, but the predominant shape was lobular

(8/11) with smooth (10/11) margins, and a homogeneous (11/11) pattern. Eleven of 12 DCIS were nonmass lesions, with a linear (7/12) or ductal (4/12) shape, and a stippled (4/12), clumped (3/12) or homogeneous (5/12) pattern. Overall, the patterns show a similar distribution to human tumor morphologies.

Discussion

In this study, we show that MR imaging techniques can be successfully applied to non-palpable, microscopic invasive and *in situ* murine mammary cancers. The importance of this accomplishment lies in the fact that (i) modeling early cancers in transgenic animals heretofore required sacrifice of the animals to assess the impact of potential therapies, and (ii) these tumors are realistic models of the most frequently detected human cancers, i.e., those early tumors that are small. We found that MRI can reliably detect the microscopic stages of both *in situ* and invasive murine mammary cancers with high sensitivity. We also found that all image-detected lesions were determined to be cancer upon pathological diagnosis. To our knowledge, this is the first report of *in vivo* MR imaging of microscopic murine mammary cancer (Arkani *et al* 2007). Abbey *et al* used PET to image DCIS and early murine mammary cancer; however, the correlation with histology was made *ex vivo* (Abbey *et al* 2004). In addition, MR imaging offers superior spatial resolution compared with PET for lesion localization and characterization. We next plan to combine the excellent anatomic detail of *in vivo* MRI with molecular imaging modalities, such as PET and optical imaging.

Our study was performed primarily to determine whether or not MR imaging of early murine mammary cancer was feasible. Since there have been no previous reports of MR imaging of murine DCIS or early invasive cancers, optimal methods for MRI of murine DCIS had not yet been developed. Therefore, we evaluated several pulse sequences and found that gradient echo (GE) images with modest T_1 -weighting (although variable due to respiratory gating) and fat suppression produced the clearest *in vivo* images of mammary glands and cancer compared with T_2 -weighted spin-echo (SE) images. To image murine mammary cancer, suppression of signal from the mammary fat pad is important, which was achieved effectively in the GE and HiSS images. The pulse sequences and parameters used in this initial study probably do not provide the best possible images of murine mammary glands. It will now be important to perform quantitative measurements of the T_1 , T_2 , T_2^* and proton density of early murine mammary cancer compared with normal tissue in order to further optimize imaging methods.

The mechanisms that produce the definitive contrast that we observed between murine mammary cancers and surrounding fibroglandular tissue and fat are not clear. Mammary glands are composed of fatty tissue, stroma and ductal/lobular structures that are lined by epithelial cells. Our results from T_1 -weighted gradient-echo and HiSS images suggest that early murine mammary cancers are conspicuous because they have both a shorter T_1 and a longer T_2^* compared to normal glandular tissue and stroma. The larger size/proton density of DCIS and small tumors compared to normal glandular tissue may also make these lesions visible. Another possibility is that fat suppression is very effective in the present

experiments because of the small field of view and the large chemical shift difference between water and fat.

Interestingly, we noted that the morphology of early murine mammary cancers on MRI is similar to the MR presentation of early human breast cancer. For example, DCIS lesions were nonmass lesions and appeared in a ductal or linear shape (Esserman *et al* 2006, Jansen *et al* 2007), while small invasive cancers appeared as round masses with smooth margins. In clinical DCEMRI of the human breast, contrast is administered to visualize the lesion. However, in the present study we used noncontrast-enhanced imaging techniques to detect early cancer, which represents a significant difference compared with clinical MRI of the human breast. This disparity may have several causes, including: (i) differences in the composition and/or distribution of glandular tissue, stroma and fat within the murine versus human mammary gland, (ii) the larger size of the murine mammary cancer relative to the whole gland when compared to human cancers, (iii) MR relaxation parameters that are significantly different in murine versus human cancers and/or normal glandular tissue or (iv) imaging at high field may yield novel contrast characteristics. Further work is needed to understand the differences between the current results in mice and those obtained in routine clinical practice. This will include precise measurements of relaxation times and proton density differences between DCIS, invasive tumors, lymph nodes and normal parenchyma in the mouse mammary glands at high field; we anticipate this will help us to determine the mechanisms underlying lesion conspicuity in our murine model. Our results also suggest the possibility of using non-contrast-enhanced techniques to image human cancers, a goal which is currently the subject of ongoing research (Du *et al* 2002, Medved *et al* 2006, Santyr 1994).

The results of the present study suggest that in the future MR imaging can be used to assess the effectiveness of therapies for cancers of all stages—*in situ*, early invasive and advanced. In addition, using the MR imaging techniques we have shown here, new contrast agents and imaging techniques that target DCIS and early invasive cancers can be developed, optimized and evaluated. DCIS is generally considered to be a precursor of invasive cancer (Recht *et al* 1998). However, because its progression cannot be routinely observed in women, the natural history of DCIS is not well understood. Evidence from studies where DCIS was initially misdiagnosed as a benign disease suggests that 14–53% of DCIS may progress to become invasive cancer (Erbas *et al* 2006). Autopsy studies have shown that DCIS is found in 5–14% of women, implying that there is a large pool of undetected DCIS in the general population (Erbas *et al* 2006). Although it is a preinvasive disease, due to the uncertainty of the natural history of individual lesions, DCIS is currently managed with obligate surgical excision (Duffy *et al* 2005). The techniques we report here provide a first step toward the use of noninvasive imaging to investigate the progression of DCIS in an animal model, and may allow us to study the characteristics of those tumors that become invasive cancers compared to those that do not. This information can be used to improve clinical management of early breast cancers.

In summary, the present study was designed to develop MR approaches to detecting early murine mammary cancer *in vivo*. We selected a transgenic mouse model with nearly 100% penetrance of mammary cancer. A logical extension of the work discussed here will be to

test our MRI detection methods of early cancers in other mouse strains that develop mammary cancer with a much lower percentage penetrance. We also plan to acquire and analyze the DCEMRI kinetic parameters of early murine mammary cancer for comparison with noncontrast-enhanced images, and are investigating the mechanism of contrast enhancement in DCIS using x-ray fluorescence microscopy to measure the distribution of Gd-DTPA (an MR contrast agent) in murine DCIS lesions. It will also be important to image thoracic mammary glands in addition to the inguinal glands reported here. However, these experiments provide proof of principle that microscopic mammary tumors can indeed be detected and followed in a mouse model of breast cancer. This is an important step toward the more effective use of non-invasive imaging in pre-clinical studies of early breast cancer.

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Figure 1.

In vitro MR images (RARE SE) with corresponding H&E stained sections of the different stages of mammary cancer. For each MR image, the display FOV is 0.8×0.48 cm. White arrowheads point to lymph nodes, thin black arrows to DCIS and thick black arrows to invasive tumors. The lymph nodes here are approximately 2–3 mm in size, while invasive tumors range from approximately 2–4 mm in size. The ducts distended with DCIS range from one to a few hundred microns in diameter. In (a) approximately $120 \mu\text{m}$ ducts with very early DCIS are detected. In (b) the ducts are now distended further with DCIS to a few hundred microns in diameter, and an area of microinvasion—i.e., where the cancer cells have penetrated through the basement membrane—is evident (thin gray arrow). This marks the beginning of the transition from *in situ* to invasive carcinoma. In (c) two relatively large ~4 mm invasive tumors are shown. In (d) smaller ~2 mm invasive tumors and DCIS are demonstrated.

Figure 2.

Axial GRE MR image of normal mammary gland (outlined in white) (a) without fat suppression, (b) with fat suppression. The display FOV is 3.0×2.0 cm. After fat suppression, signal from the mammary gland decreases. In (c) and (d), we illustrate how histology is compared to the MR image in (a). In (c), the same gland as imaged in (a) is excised (outlined in white) and the grid is visible on top, (d) the H&E section is superimposed and the grid coordinate system is noted, with z indicating the direction of the main magnetic field. The MR image in (a) represents one axial slice through the mammary gland along the z direction (in this case, $\sim z = 4$). The x dimension of the agar grid was wrapped around the mouse during imaging, and the coordinates $x = 1$ and $x = 4$ are labeled. After examining the H&E section in (d), a lymph node is identified at position $\sim (z = 4, x = 4-5)$. After examining all axial slices through the mammary gland, a structure is identified in (a) at position $(z = 4, x = 4-5)$. Thus, by relating these positions it is evident that the GRE MR image successfully detected the lymph node (white arrowheads).

Figure 3.

In vivo axial MR images (GRE with fat suppression) and corresponding H&E stained sections. The MR images and H&E stained sections represent different orientations. During imaging, the mammary glands are attached to the skin of the mouse, and are therefore wrapped around the body of the mouse. For excision, the glands are peeled back from the body of the mouse and laid flat, so that coronal H&E stained sections can be obtained. Each axial MR image represents one cross-sectional slice through the mammary gland. We used an agar grid (a polyethylene mesh embedded in partially deuterated agar, see figures 2(c) and (d)) to register the axial MR images with the H&E stained sections. (a) Lymph node (arrowhead) and DCIS (thin arrow). (b) Lymph node (arrowhead) and small tumor (thick arrow). For each MR image, the display FOV is 3.0×2.0 cm.

Figure 4.

Examples of GRE images with fat suppression of: (a) DCIS (thin arrow), (b) DCIS (thin arrow) and (c) small tumor (thick arrow). The display FOV is 3.0×2.0 cm. In (b) and (c), the agar grid is visible wrapped around the mouse.

Figure 5.

Demonstration of the same axial slice of (a) lymph node (arrowhead) and (b) DCIS (thin arrow), for three different imaging acquisitions, from left to right: GRE with fat suppression, RARE SE with fat suppression and high spectral and spatial imaging (HiSS), which yields water peak height images (shown). The display FOV is 3.0×2.0 cm. The GRE with fat suppression produced clearer images of the cancer and lymph node compared with SE. The HiSS images show the lymph node and DCIS along with excellent fat suppression.

Table 1

(a) The average signal-to-noise ratio (SNR) of muscle, normal mammary gland tissue (MGT), lymph nodes, tumors and DCIS lesions for gradient echo (GRE) images with fat suppression (FS) and RARE spin-echo (SE) images with fat suppression. (b) The average contrast-to-noise ratio (CNR) of tumors, DCIS and lymph nodes relative to muscle and normal mammary glandular tissue. Numbers are mean $\pm$ standard deviation.

(a) Average SNR						
Pulse sequence	Muscle	MGT	Tumor	DCIS	Lymph node	
GRE with FS	33.9 ± 6.0	10.3 ± 4.2	34.3 ± 12.2	30.0 ± 8.7	40.3 ± 7.4	
RARE SE with FS	26.5 ± 3.0	39.0 ± 5.5	50.0 ± 5.4	38.9 ± 8.6	44.4 ± 7.5	
(b) Average CNR						
Pulse sequence	Tumor–muscle	DCIS–muscle	Lymph node–muscle	Tumor–MGT	DCIS–MGT	Lymph node–MGT
GRE with FS	3.78 ± 6.1	−3.6 ± 8.9	6.8 ± 5.6	21.3 ± 8.3	20.6 ± 7.6	29.9 ± 6.2
RARE SE with FS	21.7 ± 5.2	13.2 ± 7.3	18.0 ± 5.9	7.08 ± 3.4	2.46 ± 5.2	5.4 ± 6.8