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MRI accurately identifies early murine mammary cancers and reliably differentiates between *in situ* and invasive cancer: Correlation of MRI with histology

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Abstract

MRI methods that accurately identify various stages of mouse mammary cancer could provide new knowledge that directly impacts management of breast cancer in patients. This research evaluates whether MRI can accurately follow the progression from *in situ* to invasive cancer, by evaluating *in vivo* and *ex vivo* MRI, and compared to histology as the gold standard for diagnosing and staging cancer. Six C3(1)SV40Tag virgin female mice between the age of 12-16 weeks were studied. At this age, these mice develop *in situ* cancer that resembles human DCIS. Fast spin echo images of inguinal mammary glands were acquired at 9.4 Tesla. After *in vivo* MRI, mice were sacrificed; inguinal mammary glands were excised and fixed in formalin for *ex vivo* MRI. 3D volume-rendered *in vivo* and *ex vivo* MR images were then correlated with histology. High resolution *ex vivo* scans facilitated comparison of *in vivo* scans with histology. The sizes of mammary cancers classified as *in situ* based on histology ranged from 150 to 400 microns in largest diameter, and average signal intensity relative to muscle was 1.40 ± 0.18 on T₂-weighted images. Cancers classified as invasive based on histology were >400 microns in largest diameter, and average intensity relative to muscle on T₂-weighted images was 2.34 ± 0.26 . Using a cutoff of 400 microns in largest diameter to distinguish between *in situ* and invasive cancers, T₂-weighted signal intensity of at least 1.4 times that of muscle for *in situ* cancer, and at least 2.3 times that of muscle for invasive cancer, 96% of *in situ* and 100% of invasive cancers were correctly identified on *in vivo* MRI, using histology as the gold standard. Precise MRI-histology correlation demonstrates that MRI reliably detects early *in situ* cancer and differentiates *in situ* from invasive cancers in the SV40Tag mouse model of human breast cancer.

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Keywords

3D MRI; Ductal carcinoma *in situ* (DCIS); Mammary intraepithelial neoplasia (MIN); Mouse mammary cancers; MRI histo-pathological correlation

Introduction

Magnetic resonance imaging (MRI) plays a critical role in the diagnosis and management of breast cancer (1-9). Early and accurate detection of breast cancer decreases morbidity and mortality. Although MRI reliably detects invasive cancers, sensitivity and specificity for DCIS is suboptimal (10-14). In addition, MRI does not reliably differentiate between DCIS that is likely to remain indolent versus DCIS that will rapidly become invasive and life-threatening (12). The processes that determine the natural history of DCIS, and in particular, the processes that trigger the progression of pre-invasive ductal carcinoma *in situ* (DCIS) to invasive breast cancer are not well understood.

The natural history of DCIS is difficult to study in women. Due to obligate surgical excision of DCIS, subsequent lesion progression cannot be studied in patients. Transgenic mouse models provide an important framework for such research in a preclinical setting. These models allow longitudinal assessment of disease development and progression. However, there have been few non-invasive imaging studies of the natural history of spontaneously occurring murine *in situ* cancers. Recently, MRI was used in this laboratory to acquire high resolution *in vivo* MR images of *in situ* cancers in the simian virus 40 large T antigen (SV40Tag) transgenic mouse model of human breast cancer (15-19). These mice develop *in situ* cancer that resembles DCIS in women (20-22) and is referred to as mammary intraepithelial neoplasia (MIN) in mice. Results from this laboratory demonstrated that these *in situ* cancers usually, but not always, progress to the invasive stage in SV40Tag mice (15, 19). Recent work from this laboratory documented a range of growth rates in both *in situ* and invasive mammary cancers, based on longitudinal MRI data (23).

Previous results from this laboratory (15, 19) showed differences between *in situ* and invasive murine mammary cancers on MRI and histology. However, sensitivity and specificity were not measured quantitatively. Earlier experiments were not designed to produce unbiased estimates of sensitivity and specificity, but instead compared a selected subset of lesions on MRI and histology (15, 19). In the work presented here, *all* lesions identified on *in vivo* MRI in the entire inguinal gland were correlated with the corresponding cancers identified on histology to allow unbiased estimates of sensitivity and specificity. The results reported here, based on precise correlation of a large number of lesions on MR and histopathology (24), demonstrate quantitatively for the first time that MRI reliably identifies early mammary cancers in SV40Tag mice, and differentiates *in situ* from invasive cancers with high sensitivity and specificity.

Materials and Methods

Ethics Statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All animal work was approved by the University of Chicago Institutional Animal Care and Use Committee (IACUC). All *in vivo* imaging studies in mice were performed under isoflurane anesthesia. All efforts were made to minimize any suffering, and after the experiments the mice were humanely euthanized.

Animal

Six C3(1)SV40 large T-antigen (Tag) transgenic female mice (FVB/N background) were used. These mice were sacrificed after *in vivo* MRI studies at 12-16 weeks of age so that both *in situ* and invasive cancers found by MRI could be correlated with histology. Mice were anesthetized prior to *in vivo* MR imaging, and anesthesia was maintained during imaging with 1-2% isoflurane. Temperature, heart rate, and respiration rate were monitored by SA instruments (Stony Brook, NY), and the respiration rate was used to gate imaging. After *in vivo* imaging, mice were sacrificed by an overdose of isoflurane and cervical dislocation.

Preparation of mammary glands for *ex vivo* MRI and histology

High resolution *ex vivo* MRI was used to facilitate correlation of *in vivo* images with histology as demonstrated previously by Xu et al. (24). After *in vivo* MRI studies, skins were excised from the mouse body and placed in 10% formalin for tissue fixation for two weeks. Prior to *ex vivo* imaging the skin was removed from formalin and rinsed daily with phosphate buffered saline (PBS, Fisher Scientific, Waltham, MA) for 3 days. A single inguinal gland from each mouse was chosen by selecting 3 right and 3 left glands from the 6 mice. The selected gland was then excised from the skin and placed between two layers of pathology foam, mimicking the exact position of the *ex vivo* mammary gland on the skin, and the exact position of the mammary gland in H&E stained slices. The gland was then placed in fomblin (Solvay Solexis, West Deptford, NJ). Plastic wrap was folded around the sample, and placed in the MRI coil for imaging. Fomblin is an oily, fluorinated polymer with no MRI signal, used to keep the tissue moist while imaging, and minimize susceptibility gradients. After *ex vivo* MRI, the sample was removed from the wrap, washed thoroughly in PBS, and put in a histology cassette in ethanol without any distortion of the tissue for immediate histological processing. Since the *ex vivo* gland was scanned by MRI in the same orientation as the hematoxylin and eosin-stained tissue, the *ex vivo* MR images served as a 'bridge' between *in vivo* MRI and histology to facilitate accurate correlation.

MRI experiments

***In vivo* imaging**—MR images were acquired on a 9.4 Tesla small animal scanner (Bruker, Billerica, MA) with 11.6 cm inner diameter, actively shielded gradient coils (maximum constant gradient strength for all axes: 230 mT/m). The mouse was placed supine on an animal holder and inserted into a 35 mm diameter quad coil (Bruker). Multi-slice RARE

(Rapid Acquisition with Relaxation Enhancement) T₂-weighted images with fat suppression were acquired with the following parameters: TR/TE_{effective}=4000/20.3 ms, field-of-view (FOV) = 25.6 mm × 25.6 mm, matrix size=256², slice thickness=0.5 mm, RARE factor=4, number of averages (NEX) = 2. In-plane resolution was 100 microns. For the inguinal glands (left and right) two interleaved sets of images were acquired to cover the slice gaps of 1 mm and then combined together for a total of 82 slices.

Ex vivo imaging—*Ex vivo* MR images were acquired on the same 9.4T scanner. The wrapped, single mammary gland prepared as described above was placed in a homebuilt 8-leg, low-pass, half-open birdcage coil (length=3 cm, width=3 cm, height=2 cm). *Ex vivo* 3D images were acquired with the RARE T₂-weighted sequence with fat suppression and with TR/TE_{effective}=4000/25 ms, FOV=26.4 mm × 22.2 mm × 6.4 mm, matrix size=384 × 320 × 64, resolution=69 microns × 69 microns × 100 microns, and NEX=2.

Histology

Following *ex vivo* MRI studies, an intact, single inguinal gland from each mouse was placed in a histology cassette and paraffin embedded. Typical tissue dimensions were 15 mm in length, 10 mm in width and about 1 - 2 mm in thickness. During pathological sectioning, tissue was removed until a full face of tissue was found. Then one 5 microns thick slice was sectioned for H&E, the next 50 microns was discarded, the next 5 microns thick slice was sectioned for histology, and the next 50 microns discarded, and so on until the tissue was exhausted. This meant that about 20-23 H&E slices were needed to cover a 1.1-1.3 mm thick slice of tissue. Histological slides were then evaluated by an experienced breast pathologist (J.M.) and tissue was classified as normal gland, *in situ*, or invasive cancers. H&E slides were then scanned using a fully automated Leica microscope (DM-6000B, Leica Microsystems, Wetzlar, Germany) for visualization and stored as images in TIFF format.

Data analysis

MRI data were processed and analyzed quantitatively using software written in IDL (Exelis VIS, Inc., Boulder, CO). To facilitate correlation of lesions found on *in vivo* MRI and histology, each mammary gland was divided into four quadrants (each quadrant approximately 3.5 mm × 2 mm), and all cancers and other features in each quadrant on *in vivo* MRI were matched with corresponding features in the same quadrant on *ex vivo* MRI and histology. The subdivision of the mammary glands into quadrants added an extra check to reduce errors in correlation. The quadrants in each gland were labeled as top, mid-top, mid-bottom, and bottom. The high resolution *ex vivo* scans were examined to insure that corresponding lesions on *in vivo* scans and histology were chosen. Identification was confirmed by two observers – one (D.M.) with 10 years of experience in MRI and one (E.M.) with 10 years of experience in histology. The person who classified the cancers as *in situ* or invasive cancer based on MRI was blinded to histopathology assessments.

An integrated software program, Amira (FEI Visualization Sciences Group, Burlington, MA), was used for volume rendering and 3D visualization of *in vivo* images. This program rotated the *in vivo* MR images into the same orientation as *ex vivo* MR and histology images

for precise MRI-histopathology correlations. This allowed correlation of all lesions identified by MRI in the entire gland with histology.

The signal-to-noise ratio (SNR) over regions-of-interest (ROIs) in muscle, parenchyma, *in situ* and invasive cancers was calculated by selecting axial slices containing these tissue types. The ROIs for data analysis were drawn based on MR images without prior knowledge of histology. The average SNR in ROIs of each tissue type was then divided by the average SNR in muscle. Average values with standard deviations were calculated for parenchyma, *in situ* and invasive cancer. Two-way ANOVA was performed between the tissue types for statistical analysis. A p-value <0.05 was considered significant.

Results

The general anatomy of mammary glands of a 14 week old SV40 female mouse is shown on axial T₂-weighted images in Figure 1. The lymph node is clearly seen in the axial slice shown in Figure 1a1. Figure 1b1 shows a second axial slice 2 mm inferior to the end of lymph node. Figures 1a2 and 1b2 show only the left glands in color, from the same axial slices, segmented from the rest of the mouse and expanded so that the mammary gland anatomy can be easily appreciated. In Figure 1b2 three small *in situ* cancers of about 183-250 microns in diameter are shown and indicated by red arrows. These cancers were correlated with higher spatial resolution (69 microns in-plane resolution) *ex vivo* images and with histology (not shown in this figure). *In situ* cancers of this size generally appeared at week 14. Figure S1 shows a movie drawn from 35 axial slices and these slices are illustrated in sequence. Figure S2 shows a movie of the 3D volume rendered image of the mouse inguinal gland, drawn from 35 axial slices, as illustrated in sequence as in Figure S1. Figure S3 shows a movie of the 3D volume rendered image of the inguinal mammary gland inside the mouse abdomen. For Figures S1-S3 see “Supplementary Materials.”

Based on the methods described above, suspicious lesions found on *in vivo* and *ex vivo* MRI were reliably correlated with histology. Figure 2 demonstrates precise correlation of *in vivo*, *ex vivo* MR, and histological images. Figure 2a compares 3D volume rendered images from *in vivo* (left) MRI with *ex vivo* (right) MRI of the same mammary gland, excised from a 12 week old SV40 mouse. The mammary gland lies in a circular position extending from the abdomen to the back. To compare the *in vivo* and *ex vivo* glands, the *ex vivo* images were rotated in 3-dimensions so that they could be compared to the *in vivo* orientation. In both images in Figure 2a, the lymph node is indicated and labeled. Seven suspicious lesions are indicated by color coded arrows on the 3D *in vivo* and *ex vivo* images. The apparent position of the invasive cancer in the *in vivo* image is not identical to the position in the *ex vivo* image (shown by red arrows in Figure 2a). This is because the excised glands in *ex vivo* images are flat – while the mammary glands *in vivo* curve around the mouse's body. When the mammary gland is excised and flattened, the large invasive cancer shown with the red arrow unfolds onto the position indicated by the corresponding red arrow on the *ex vivo* image.

Figure 2b compares an *ex vivo* MR image with an H&E image. Since only one H&E stained slice is shown – rather than all slices taken from the entire mammary gland – only a subset

of these suspicious lesions can be found on the histology slice. A total of seven masses are identified. One is invasive cancer, as indicated by red arrows in all four panels. In Figure 2b, four clearly visible masses near the lymph node are unambiguously identified and they are matched with color-coded arrows in both *ex vivo* MR and H&E images. Based on histology three of these lesions were classified as *in situ* cancer and one was classified as invasive cancer, above the lymph node as indicated by a red arrow in Figure 2b. The *ex vivo* images show that the corresponding lesions on *in vivo* images and histology are correctly identified, so that suspicious lesions on *in vivo* images can be correctly classified as *in situ* or invasive using histology as the gold standard.

Figures 3 and 4 show correlation of *in vivo* MRI, *ex vivo* MRI, and histological images for 14 and 16 week old SV40 mice. In Figures 3a and 4a we compare *in vivo* 3D volume rendered MR images (left) with the *ex vivo* MR images of the same mammary gland (right). Similarly, Figures 3b and 4b compare the corresponding *ex vivo* MR with H&E images. The clearly visible masses were classified as *in situ* and/or invasive cancers based on histology, and the corresponding lesions on MR and H&E were unambiguously identified on histology and *in vivo* MRI with the help of the *ex vivo* images. Figure 4b illustrates the use of quadrants to insure that the corresponding cancers on MRI and histology were reliably identified. On the histology image in Figure 4b, four quadrants are defined as ‘top’, ‘mid-top’, ‘mid-bottom’ and ‘bottom’. For correlation lesions found in *in vivo* MRI and histology were matched in each of these four quadrants. The movie in Figure S4 shows a stack of H&E images of a single inguinal mammary gland from a 16 week old C3(1)SV40Tag mouse (see “Supplementary Materials”).

Figure 5 shows an expanded comparison of *in vivo* and *ex vivo* MR images with an H&E image of a 12 week old SV40 mouse – only the left inguinal gland is shown here. The location of an invasive cancer is indicated by a red arrow in each image. The distance between the center of the invasive cancer and the center of lymph node was 1.96 ± 0.10 mm, 1.91 ± 0.07 mm, and 1.98 ± 0.06 mm from *in vivo*, *ex vivo* MR, and histology images, respectively. The diameter of the invasive cancer was 0.710 ± 0.020 mm, 0.707 ± 0.018 mm, and 0.713 ± 0.011 mm from *in vivo*, *ex vivo* MR, and histology images, respectively. The values were in excellent agreement with less than 5% difference between the measurements on *in vivo*, *ex vivo* MR, and histology. As seen on H&E stained tissue shown in Figure 5c, pink areas surrounding the cancerous mass are extended stromal cells as assigned by a breast pathologist (J.M.). The brighter area at the top of the invasive cancer is distinctly visible in the *ex vivo* image in Figure 5b, but is less obvious in the *in vivo* image (Figure 5a).

A total of six glands in six mice were evaluated. Based on the correlation with histology detailed above, a total of 58 cancers were found. Of these, 52 were *in situ* cancers and 6 were invasive. The largest diameter of *in situ* cancers (measured in the axial plane) ranged from 0.183-0.385 mm on MRI; this was in good agreement with the range of sizes found on H&E, which was from 0.175 to 0.379 mm. The average signal intensity of *in situ* cancers relative to muscle on T₂-weighted images (TR/TE = 4000/20 ms) was 1.40:1, as listed in Table 1. Invasive cancers ranged in largest diameter from 0.45 to 1.8 mm on MRI, in good agreement with diameters on H&E. Average signal intensity of invasive cancers relative to muscle on T₂-weighted images was 2.34:1, as listed in Table 1.

The sizes and relative signal intensities identified based on correlation of MRI with histology were evaluated as markers for cancer and for differentiating between *in situ* from invasive cancer. We tested whether lesions identified on MRI with largest diameter between 0.18 and 0.38 mm, and signal intensity relative to muscle between 1.22 and 1.58 could reliably be identified as *in situ* cancer. In addition, we tested whether lesions identified on MRI with largest diameter greater than 0.4 mm and signal intensity relative to muscle of at least 2.08:1 could reliably be identified as invasive cancer. Lymph nodes could be identified and excluded from this analysis based on morphology (17, 19), signal intensity (25), and location. Based on these criteria; for *in situ* cancer there were 50 true positives (TP) i.e. 96.2% of *in situ* cancers were correctly identified and classified by MRI, four were false positive (FP), and two were false negative (FN). For invasive cancers there were six true positives – all invasive cancers were correctly identified. We did not observe any benign masses in this study. The results for all cancers found in each mouse are summarized in Table 2.

Discussion

The goal of this work was to evaluate the accuracy of MRI in identifying mammary cancer and differentiating *in situ* from invasive cancer. This was achieved by correlating *in vivo* MRI of mouse mammary glands, *ex vivo* MRI, and histology. The results demonstrate that high resolution *ex vivo* MRI can serve as a bridge to facilitate correlation of *in vivo* MRI with histology. Because the *ex vivo* images have approximately the same orientation as the fixed tissue, they show clearly the correspondence between features on MRI and histology. Figure 4 demonstrates that in each quadrant of the mammary gland, cancers on *in vivo* and *ex vivo* MRI and histology were accurately identified based on their location within the quadrant and their location relative to lymph nodes and other landmarks.

In vivo MRI was able to reliably identify cancers and accurately distinguish between *in situ* and invasive cancers. This demonstrates that *in vivo* MRI has adequate resolution and contrast to identify *in situ* cancers at an early stage and follow their development and transition to the invasive phenotype. As a result, serial MRI can be used in mouse models to non-invasively evaluate effects of experimental neo-adjuvant therapies and chemo-prevention on the development of early mammary cancers. This new information from MRI could accelerate the development of effective new treatments.

The present results demonstrate that *in situ* and invasive murine mammary cancers can be reliably differentiated simply on the basis of size and signal-intensity on spin echo images relative to muscle. *In situ* cancers contain a combination of normal ductal tissue and cancer growing in the ducts; therefore it makes sense that signal intensity in *in situ* cancers is intermediate between normal ducts and invasive cancers. Using size and signal intensity together reduced the number of false positives/negatives for *in situ* cancers. The number of lesions incorrectly identified as *in situ* cancers based on size alone was small but significant – 8-10%. When signal intensity was added as a criterion, all invasive cancers were correctly identified.

Use of size and T₂-weighted signal intensity as markers for cancer stage will facilitate studies of cancer initiation and progression in the SV40 Tag mouse model of breast cancer. Moreover, these simple size and intensity markers can be used in this transgenic mouse model as 'gold standards' to guide the development of functional image-based biomarkers that are useful in the clinical setting. For example, quantitative parameters derived from diffusion-weighted imaging, dynamic contrast enhanced MRI, or quantitative spin echo imaging can be tested in mouse models using size and signal intensity as gold-standards.

Signal intensity on T₂-weighted images was important for distinguishing *in situ* from invasive cancer. However, in the present experiments, this may have been affected by tumor size. Small *in situ* cancers may often have been thinner than the imaged slice (0.5 mm) and thus partial volume effects may have decreased the measured signal intensity. In future studies, higher spatial resolution in T₂-weighted images combined with quantitative T₂ measurements may improve characterization of *in situ* cancers.

In situ cancers were reliably identified in the present study in part because the signal-to-noise ratio of normal parenchyma was very low (Table 1). Normal mammary ducts in the mice used in this study are 20 - 50 µm in diameter, have a relatively short T₂/T₂* (25), and are not easily detected on T₂-weighted images. As a result, small, *in situ* cancers 150-400 µm in diameter were easily detected against a relatively dark background. While signal from the parenchyma did not interfere with cancer detection in this mouse model, it is not known at present whether this also applies to other mouse models that might have denser parenchyma.

Because *ex vivo* MR images could be acquired with very high spatial resolution, they served as a bridge between *in vivo* MRI and histology to facilitate more reliable correlation of each feature on MRI with the corresponding feature on histology. In addition, visualization of the entire mammary gland in 3D using Amira software helped us to make the correct identifications and to determine the positions of *in situ* and invasive cancers. Reliable comparisons were also aided by the fact that cancers are relatively sparse in the SV40 Tag model with no more than two cancers in each quadrant. In mouse models with denser cancers more sophisticated methods for precise correlation using fiduciary markers may be needed (17).

Conclusions

Precise correlation of *in vivo* and *ex vivo* MRI with histology in the widely used C3(1)SV40Tag murine model of breast cancer demonstrates for the first time that MRI can detect early murine mammary cancers with high sensitivity and specificity and accurately distinguish between *in situ* and invasive cancer. As a result, MRI can be used to non-invasively identify early mammary cancers *in vivo* and follow the progression from ductal carcinoma *in situ* to invasive cancer in a transgenic mouse model. Based on the sizes of *in situ* and invasive cancers and their signal intensities on T₂-weighted MR images, 96% of *in situ* cancers and 100% of invasive cancers were accurately identified on *in vivo* MRI. This provides a basis for further work to identify functional and anatomic image-based markers that can be used clinically to distinguish aggressive from indolent cancers. The results

presented here suggest that non-invasive MRI studies of mouse models can increase our understanding of the transition from *in situ* to invasive cancer, and the effect of neo-adjuvant and preventive therapy on this transition. This could have a significant impact on the use of MRI in the clinical management of breast cancer and could also guide the development of new breast cancer therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

ANOVA	analysis of variance
DCIS	ductal carcinoma <i>in situ</i>
FN	false negative
FP	false positive
FOV	field of view
H&E	hematoxylin and eosin

MIN	mammary intraepithelial neoplasia
NEX	number of excitation
RARE	rapid acquisition with relaxation enhancement
ROI	region-of-interest
SNR	signal-to-noise ratio
SV40 mouse	C3(1)SV40 the simian virus 40 large T antigen (Tag) transgenic female mouse
TP	true positive

Figure 1.

In vivo T₂-weighted MR images of a 14 week old C3(1)SV40Tag mouse are shown. Two MR slices, 2 mm apart are shown in panels a1 and b1. MRI parameters are TR/TE of 4000/20 ms, FOV of 25.6 mm × 25.6 mm, array size of 256 × 256, slice thickness of 0.5 mm with 2 averages. Panels a2 and b2 show MR images (in color) of the corresponding left glands only. The lymph node is labeled in a2. Three small *in situ* cancers, 183-250 μm in largest diameter, are indicated by red arrows; a clearly visible abdominal ligament is indicated by a yellow arrow in panel b2. Regions-of interest for muscle are indicated by yellow circles. Scale bars are shown in all images.

Figure 2.

Correlation of *in vivo*, *ex vivo* MR and histological images of a 12 weeks old SV40 female mouse is illustrated. Panel a compares *in vivo* (left) and *ex vivo* (right) 3D volume rendered images – only the left mammary gland is shown here. *In vivo* 2D images were acquired with a T_2 -weighted sequence with TR/TE of 4000/20 ms, FOV of 25.6 mm, array of 256×256 , slice thickness of 0.5 mm, in-plane resolution of 100 microns, and 2 averages. *Ex vivo* 3D images with the excised mammary gland were acquired using the same T_2 -weighted sequence with TR/TE of 4000/25 ms, FOV of $26.4 \text{ mm} \times 22.2 \text{ mm} \times 6.4 \text{ mm}$, array of $384 \times$

320 × 64, resolution of 69 microns × 69 microns × 100 microns, and 2 averages. In both images (left and right panels), the lymph node is identified and labeled; regions of cancers are also identified and matched with color-coded arrows in *in vivo* and *ex vivo* images. The images are rotated in 3-dimensions so that they are roughly in the same orientation. Panel b compares an *ex vivo* MR image (left) with an H&E image (right). A central slice from the *ex vivo* MR images of the excised mammary gland is shown here – the lymph node (LN) and the brighter spots for *in situ* and invasive cancer around LN are indicated by color arrows; the same color-coded arrows are also shown in H&E image to co-register regions of *in situ* and invasive cancer with the *ex vivo* MR image. A scale bar of 2 mm in both images of panel ‘b’ is shown. The red arrow in each image indicates invasive cancer; other arrows indicate *in situ* cancer.

Figure 3.

Correlation of *in vivo*, *ex vivo* MR and histological images of a 14 week old SV40 female mouse is shown. Panel a compares *in vivo* (left) and *ex vivo* (right) 3D volume rendered images – only the left mammary gland is shown here. MRI parameters were described in Figure 2. In both images, the lymph node is identified and labeled; regions of *in situ* cancer are also identified and matched with color-coded arrows in both sets of images. Panel b compares an *ex vivo* MR image with an H&E image. A central slice from the *ex vivo* MR images of the excised mammary gland is shown here – the lymph node and the other clearly

distinct *in situ* cancers around the lymph node (LN) are indicated by color arrows; color-coded arrows show corresponding cancers on H&E and the *ex vivo* MR image.

Figure 4. Correlation of *in vivo* MRI, *ex vivo* MRI and histological images of a 16 week old SV40 female mouse is shown. Panel 'a' compares *in vivo* (left) and *ex vivo* (right) 3D volume rendered images – only the right mammary gland is shown here. MRI parameters were described in Figure 2. In both images in 'a', the lymph node is identified and labeled; regions of invasive cancers and *in situ* cancers are also identified and matched with color-coded arrows in both sets of images. Panel 'b' compares an *ex vivo* MR image with an H&E image. A central slice from the *ex vivo* MR images of the excised mammary gland is shown

here – the lymph node and the other clearly distinct mammary cancers are indicated by color arrows. One mass in panel ‘a’, as indicated by white arrows, is not seen in the central slices of *ex vivo* MR and H&E images. Otherwise, the same color-coded arrows are also shown in H&E image to co-register regions of mammary cancers with that of *in vivo* and *ex vivo* MR image. On the histology image in panel ‘b’, the dashed lines are shown and four quadrants are labelled as top, mid-top, mid-bottom and bottom – for correlation we matched lesions found in *in vivo* MRI and histology using these four quadrants.

Figure 5.

MRI-histopathological correlation of mammary cancers in a mouse model is illustrated. Panel 'a' shows an *in vivo* MR image of a 12 week old SV40 female mouse – only the left inguinal mammary gland is shown here. Panel 'b' shows an *ex vivo* image in the proximity of the lymph node of the excised mammary gland and compares with an H&E image, shown in panel 'c'. *In vivo* and *ex vivo* MRI parameters were as in Figure 2. The lymph node is

labeled in 'b' and 'c'; red arrows indicate the location of an invasive cancer. A scale bar is indicated in each image.

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Table 1

Signal-to-noise ratios (SNR) of *in situ* and invasive cancers. Average ($\pm$ standard deviations) SNR for various tissue types¹ are compared to SNR in muscle in SV40 female mice (n=6) of 8-16 weeks of age.

Parenchyma	<i>In situ</i>	Invasive cancer
0.63 $\pm$ 0.14	1.40 $\pm$ 0.18	2.34 $\pm$ 0.26

¹To measure SNR of the parenchyma, we used mice at 8 weeks of age when no apparent *in situ* cancer was seen. To measure the SNR of *in situ* and invasive cancers, we used mice at 12-16 weeks of age as seen in Figures 3, 4 and 5. Values of SNR were compared between these three groups by two-way ANOVA and the differences were found to be statistically significant ($p < 0.006$).

Table 2

Correlation of *in situ* and invasive cancers on *in vivo* MRI and histology images. The numbers of *in situ* and invasive cancers in SV40 female mice (n=6) of 12-16 weeks of age are listed.

Cancer type ¹	H&E	<i>in vivo</i> MRI	TP ²	FP ²	FN ²
<i>In situ</i> cancer	52	54	50	4	2
Invasive cancer	6	6	6	0	0

¹For correlation we matched lesions found on *in vivo* MRI and histology using four quadrants of 3.5 mm × 2 mm in size, labeled as top, mid-top, mid-bottom, and bottom, as indicated in Figure 4.

²True positive (TP) lesions were identified in both *in vivo* MR images and histological images; False positive (FP) lesions were identified only in *in vivo* MR images and the corresponding lesions were not found in histological images; False negative (FN) lesions were identified only in histological images and the corresponding lesions were not identified in *in vivo* MR images.