# 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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**Target Audience**: The study will benefit both pre-clinical and clinical investigators. It will allow investigators to develop better MRI-based markers for tumor progression, improve understanding of cancer initiation and progression, evaluate response to therapy in murine models of breast cancer, and provide valuable insights regarding clinical management of patients with early breast cancers.

**Purpose**: MRI can play a critical role in studies of mouse models of breast cancer. MRI methods that accurately identify various stages of mouse mammary cancer can provide new knowledge that directly impacts management of breast cancer in patients. This research tests whether MRI can accurately follow the progression from ductal carcinoma *in situ* to invasive cancer by evaluating *in vivo* MRI and *ex vivo* MRI, and comparing these images to histology as the gold standard for diagnosing and staging cancer.

**Methods**: C3(1)SV40Tag virgin female mice (n=12) were studied. This mouse model develops *in situ* cancer that resembles human ductal carcinoma *in situ*. Six mice were MR imaged serially every other week between 8 and 22 weeks of age during the initiation of *in situ* cancer and progression to invasive cancer. Another six mice were imaged at 12, 14, and 16 weeks of age (n=2 per age group) and then sacrificed immediately. After sacrifice, inguinal mammary glands were excised and fixed in formalin for *ex vivo* MRI. MR images were acquired with a 9.4 Tesla Bruker scanner. Cancers were identified on *in vivo* and *ex vivo* fast spin echo images ('RARE'; TR/TE=4000/20 ms). High resolution *ex vivo* scans were used to facilitate correlation of *in vivo* scans with histology, and to check for very small *in situ* cancers that were not detectable on *in vivo* scans. 3D volume-rendered *in vivo* and *ex vivo* MR images were then correlated with histology. The person who classified the cancers on MRI was blinded to histopathology assessments.

**Results and Discussion**: Normal mouse mammary ducts (20-50 μm in diameter) have a relatively short $T_2/T_2^*$, and are not easily detected on $T_2$-weighted MRI. At 12 weeks of age, denser parenchyma is detected. Small masses (150-400 μm in diameter) with high signal intensity relative to muscle on $T_2$-weighted images were identified as *in situ* cancer based on histology (Figure 1). Masses of >400 μm in diameter, with even higher signal intensity were identified as invasive cancer based on histology. Signal intensity on $T_2$-weighted images in the parenchyma, *in situ* and invasive cancers relative to muscle was 0.63 ± 0.14, 1.40 ± 0.18 and 2.34 ± 0.26, respectively ($p<0.006$). 96% of *in situ* and 100% of invasive cancers identified on *in vivo* MRI agreed with histology (Table 1).

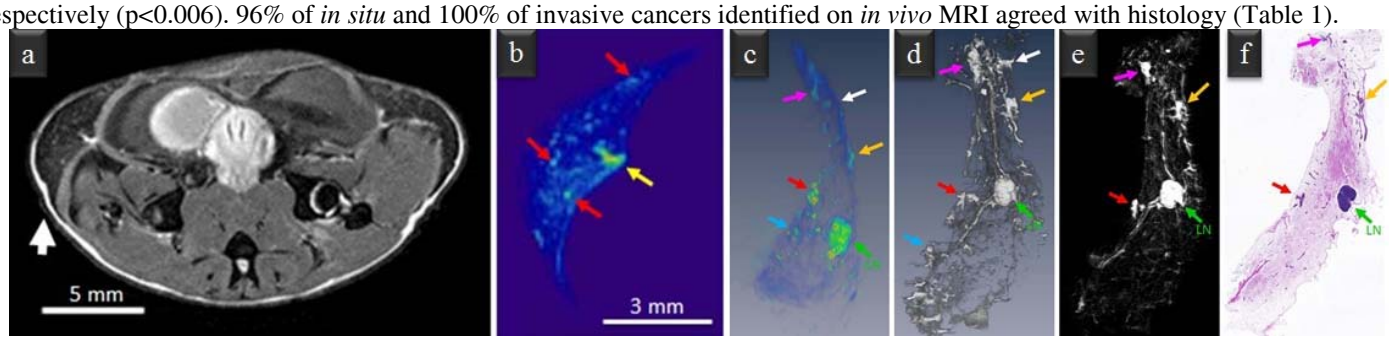


**Figure 1. Correlation of *in vivo*, *ex vivo* MR and histological images of a SV40Tag mouse**. An axial $T_2$-weighted MR image of a 14 week old SV40 mouse is shown in **a**; the left mammary gland is indicated by a white arrow. The corresponding left mammary gland in color is shown in **b**; three small *in situ* cancers are indicated by red arrows; a clearly visible abdominal ligament is indicated by a yellow arrow. Panels **c** and **d** compare *in vivo* and *ex vivo* 3D volume rendered images of the left gland, respectively, of the same mouse. Panels **d** and **e** compare an *ex vivo* MR image (**e**) with an H&E image (**f**) – a central slice from the excised mammary gland in each set 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 MR images.

**Table 1**. **Co-registration of *in situ* and invasive cancers on *in vivo* MRI and histology images**. The total numbers of *in situ* and invasive cancers in SV40Tag mice as found in *in vivo* MRI and histology are listed. Six SV40Tag virgin female mice between the age of 12 and 16 weeks were used.

| SV40 Mice | *In situ* Cancer | | Remarks[a] | | | Invasive Cancer | | Remarks[a] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | H&E | *in vivo* MRI | TP | FP | FN | H&E | *in vivo* MRI | TP | FP | FN |
| **n=6** | **52** | **54** | **50** | **4** | **2** | **6** | **6** | **6** | **0** | **0** |

[a]True 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.

**Conclusions**: Precise correlation of MRI with histology demonstrates for the first time that MRI can detect early murine mammary cancers with high sensitivity and specificity, follow the progression and accurately distinguish between *in situ* and invasive cancer in a transgenic mouse model of human breast cancer. 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 present results also suggest that non-invasive serial MRI studies of mouse models can increase our understanding of the effects of preventive therapy. This will have a significant impact on the use of MRI in the clinical management of breast cancer and the development of new breast cancer therapies.