Follow-up study of *in vivo* breast cancer cell invasion by MRI

R. Lemay[1], C. Pépin[2], L. Tremblay[2], B. Paquette[1], and M. Lepage[2]

[1]Dép. de médecine nucléaire et de radiobiologie, Université de Sherbrooke, Sherbrooke, QC, Canada, [2]Centre d'imagerie moléculaire de Sherbrooke, Université de Sherbrooke, Sherbrooke, QC, Canada

Introduction: The majority of women with stage I and II breast cancer are preferentially treated by breast-conserving surgery followed by radiation therapy.[1] Although radiotherapy is associated with an important reduction of local recurrence five years following treatment, only a moderate increase of survival rate is observed 15 years after treatment.[2] Epithelial breast cells must attach to the basement membrane in order to differentiate and proliferate. Our aim was to determine whether irradiation of a tissue would increase the invasiveness of tumor cells injected subcutaneously post-irradiation.

Method: One thigh of Balb/c mice was irradiated (30 Gy with a Gamma Knife, LEKSELL ELEKTA™) using 14-mm collimators while the second thigh only received background irradiation (< 0.3 Gy) and was used as a control. Mammary carcinoma MC7-L1 cells[3] (10^7) were subsequently injected subcutaneously in both thighs. T_1-weighted dynamic contrast-enhanced MRI experiments were conducted three times during the interval from 3 to 6 weeks after the injection. All animals were anaesthetized with isoflurane and their body temperature maintained at 37°C using a warm air blower controlled by a rectal thermistor feedback. Mice were placed in a 40-mm Millipede™ RF probe inside a Varian 7T scanner. Fifty consecutive sets of gradient-echo images were acquired with the following parameters: TR/TE = 100/2.49 ms, matrix size 128 x 128, FOV 30 x 30 mm^2, 10 slices of 1.5 mm, NA 4, and a 30° flip angle. After the third set, Gd-DTPA (180 µl, 1:2.5) was injected i.v. via the tail vein. An automated analysis of the sets of images based on the signal intensity difference between normal muscle tissue and tumor tissue segmented the images to reveal the extent of tumor infiltration. To confirm the MRI findings, tumors and nearby tissues were excised, fixed in 10 % buffered formalin, embedded in paraffin, sectioned in 4-µm cross-sections, stained with hematoxylin and eosin (H/E) and observed by light microscopy.

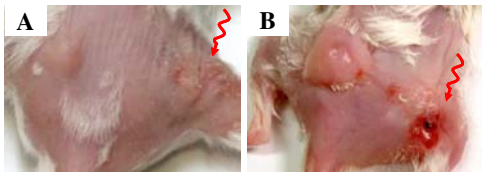

Fig. 1. Pictures of tumor-bearing mice (A) 3 weeks and (B) 6 weeks after inoculation of MC7-L1 cells (left thigh) or after a 30-Gy irradiation followed by inoculation of cells (right thigh). Irradiated thighs (30 Gy) are indicated by wavy arrows.

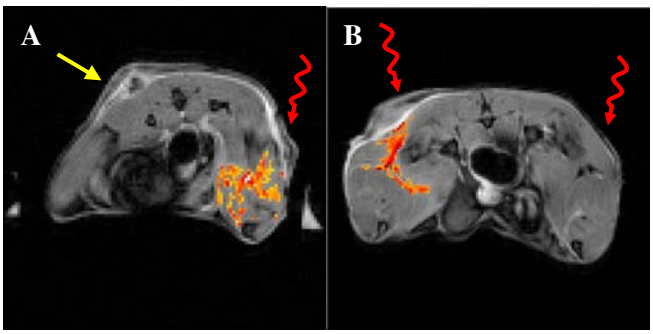

Fig. 2. Contrast–enhanced axial MR images showing invasion *in vivo* 30 minutes after an i.v injection. of Gd-DTPA. (A) Mouse 3 weeks after inoculation of MC7-L1 cancer cells on an unirradiated thigh (left, yellow arrow) or after irradiation at 30 Gy (right thigh). (B) A different mouse 6 weeks after a 30-Gy irradiation on both thighs followed by inoculation of cells (left thigh only). Irradiated thighs (30 Gy) are indicated by red arrows. The infiltrated tumor is highlighted by colored pixels.

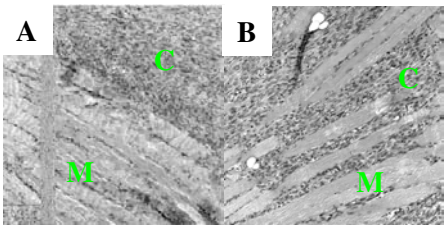

Fig. 3. Histological evaluation of excised mice tumors showing invasion *in vivo* (A) 6 weeks after inoculation of MC7-L1 cells and (B) after a 30-Gy irradiation followed by inoculation of cells.

Results and discussion: Invasion by MC7-L1 cancer cells could be observed at the macroscopic level. Tumors have propagated into the muscle region three weeks after the implantation of cancer cells on the irradiated thighs (Fig. 1, right tumor). Conversely, the same cells have only grown under the skin surface (Fig. 1, left tumor) on control, unirradiated thighs. These distinct patterns of tumor growth were more pronounced on the 6th week (Fig. 1B). The radiation-enhancement of *in vivo* tumor invasion into the muscle was monitored by MRI. Contrast-enhanced images clearly indicate the control tumor remained subcutaneous (Fig. 2A, left side) while cancer cell invasion and growth into the muscle were observed 3 and 6 weeks after irradiation (Fig. 2A, right side and B left side). Radiation without cell inoculation did not alter the MRI signal (Fig. 2B, right side, 6 weeks), supporting that detection of invasion by MRI correlated with cancer cell invasion. Histological evaluation confirmed these results. A notable infiltration by breast cancer cells (C) through muscular fibers (M) in the irradiated mouse thigh was observed (Fig. 3B), whereas no invasion was detected in the unirradiated thigh (Fig. 3A). The volume of the tumors varied widely between individuals. For the mouse on Fig. 2A, the volume of the infiltrating tumor was 77 mm^3, which can be compared to a volume of 42 mm^3 for the mouse on Fig. 2B. Overall, the results are supported by *in vitro* irradiation of basement membrane where an increased invasiveness of breast cancer cells was observed and explained by the release and activation of certain matrix metalloproteinases (not shown).

Conclusion and perspectives: We demonstrated that radiation, preceding cancer cell injection, can increase the invasion efficacy of mammary cancer cells *in vivo*. MRI allowed to visualize and to quantify the volume of infiltrating tumor within muscle tissue.

Acknowledgments: We thank Mélanie Archambault and Nathalie Picard for their technical assistance. This work was supported by NCI-CBCRA grant 13434.

References: **1**-Hiraoka *et al*., Breast Cancer **4,** 127-133 (1997). **2**- Clarke *et al*., Lancet **366**, 2087-2106 (2005). **3-** Lanari *et al*., Cancer Res. **61,** 293-302 (2001).