The Impact of Autologous Fat Grafting on Breast Cancer: An Experimental Model Using Magnetic Resonance Imaging

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ABSTRACT: **Background:** Although fat grafting is a common technique to repair defects after breast cancer reconstruction surgery and has a low complication rate, the relation between fat grafting and the risk of breast cancer is unknown. Clinical trials to investigate this connection can elucidate the benefits and potential risks of fat grafting in oncology patients.

Objectives: To establish an efficient experimental model, using magnetic resonance imaging (MRI) scans, for comparing different breast tumor study groups post-fat grafting.

Methods: Breast tumor cells were injected into immunocompromised mice. After tumors formed they were removed. Liposuction was performed in a female human donor and fat was collected. Cells were extracted from the fat by enzymatic digestion. Immunocompromised mice were randomized into four groups: a preliminary experiment group and three equal groups according to the type of fat graft: (i) fresh fat enriched with adipose-derived mesenchymal stem cells (AdMSCs), (ii) fresh fat without cell enrichment, and (iii) no fat injected. Tumor volume was assessed by serial MRI scans.

Results: The rate of tumor growth was higher in the enriched fat group compared to the non-enriched fat group.

Conclusions: This experimental model is an effective measurable method, allowing future investigation of the effect of autologous fat on breast cancer.

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KEY WORDS: fat grafting, fat transfer, adipose-derived stem cells, breast cancer, breast reconstruction

Stem cells have gained popularity due to their potential use in regenerative medicine [1] and their various interactions with the microenvironment of the recipient tissue [2]. However, some evidence of unfavorable side effects, such as tumor growth enhancement [3-5], has raised concern regarding their safety.

In plastic surgery, fat grafting has major clinical applications for cosmetic as well as oncology patients [6]. Fat grafting has become a standard of care in the repair of volume and contour defects after ablative or reconstructive surgery [7,8], although its association with the possible recurrence of breast cancer is not yet clear [9]. The hypothesis that grafted fat promotes neoplastic activity is based on the fact that adipocytes might accelerate tumor growth [10,11].

To the best of our knowledge this is the first study to assess the influence of grafted fat on the rate of tumor growth. We undertook this study to establish an experimental model to examine the hypothesis that breast cancer recurrence is influenced by grafted fat or grafted fat enriched with mesenchymal progenitor cells.

MATERIALS AND METHODS

The study was approved by our institutional review board (IRB) in accordance with the Helsinki Declaration (June 1964) and subsequent amendments. A fat-donor signed an informed consent. Animal procedures were approved by the IRB for studies in animals in full compliance with local, national, ethical, and regulatory principles, and local licensing arrangements.

FAT HARVESTING AND PROCESSING

Fat was harvested from a single female donor who underwent standard elective tumescent liposuction as previously described [12]. To eliminate inter-donor variability between recipient animals, harvested fat from only one donor was used. Forty-five female SCID mice were randomized into three groups according to the type of fat graft: (i) fresh fat enriched with adipose-derived mesenchymal stromal cells (AdMSCs), (ii) fresh fat without cell enrichment, and (iii) no fat graft (control group).

ADMSCS EXTRACTION

Cell extraction from lipo-aspirates of the same donor was performed by gentle enzymatic digestion as previously described [13], with several modifications. Prior to digestion, tissue samples were washed with phosphate-buffered saline and incubated at 37°C with a digestion enzyme mixture (trypsin-EDTA 0.25% and 2.4 U/ml dispase II, Sigma, St Louis, MO, USA) for several cycles

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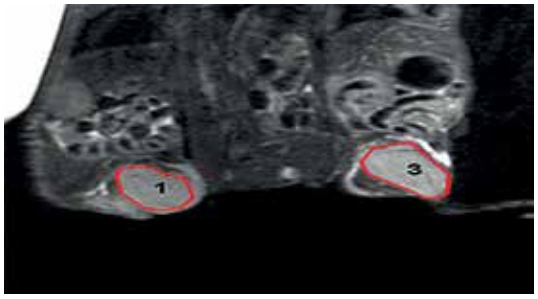
Figure 1. MRI image demonstrates implanted tumor. The tumor's volume was calculated by extrapolation from region of interest in subsequent scans



of 30–45 minutes. The cells were then added, prior to injection, to the grafted fat and mixed manually in a 10 ml syringe.

ANIMALS

Forty-five female SCID mice were divided into three groups; the recipient site was the subcutaneous space in the right thigh.

TUMOR INJECTION PROCESS

Under general anesthesia, using a 0.1 ml syringe and 27 g needle, 2 x 10^6 breast tumor cells (MDA231) were injected to the hypodermis layer of the mouse's right thigh.

TUMOR REMOVAL PROCESS

Under general anesthesia, an incision of the epidermis, dermis and hypodermis was performed along the thigh above the palpated tumor. The tumor was separated from its environment while maintaining the surrounding tissues, followed by skin closure with a continuous suture (PDS 4-0).

FAT GRAFTING

Under general anesthesia, using a 2 ml syringe and 18 G needle, fat was injected to the hypodermis layer of the thigh, into the area of the tumor bed.

VOLUMETRIC ASSESSMENT

Magnetic resonance imaging (MRI) scans were performed in all animals when the tumor reached clinical size, after tumor removal, immediately after fat grafting, 1 week after fat grafting, and 2 weeks after fat grafting. One mouse from the AdMSC enrichment group underwent another MRI scan 3 weeks after fat grafting.

MRI DATA ACQUISITION

Assessment of tumor and fat grafting volumes was performed under general anesthesia using a clinical 1.5 T MR system (General Electric, USA) and a phased-array head coil. Fast-spin echo T1-weighted MR images were acquired in the sagittal direction with the following parameters: echo time 9.8 ms, repetition time 480 ms, slice thickness 2 mm with no gap, field of view 28 x 16 cm, matrix 384 x 256 pixels, band width 31.25 KHz, 4 repetitions.

DATA ANALYSIS

The tumor volume was calculated from the MR images by plotting regions of interest to cover the entire tumor region in each slice (appearing bright on T2-weighted MR images). The number of pixels in the regions of interest was counted; then the slices were counted and multiplied by the volume of a single pixel.

STATISTICAL ANALYSIS

All results are presented as mean ± SD. Differences in volume as measured in MRI scans were expressed as a percentage of the initial volume at each checkpoint and were analyzed using ANOVA for each checkpoint; the ANOVA test was also used in repeated measures for the behavior of each group over time.

RESULTS

The group that received fresh enriched fat showed a mean weekly increase in tumor volume of 2.6 ± 1.5. The group that received fresh graft non-enriched fat showed a mean weekly increase in tumor volume of 2.4 ± 0.04. The control group did not show any changes in tumor volume throughout the study. The weekly growth pace of tumor volumes in the fresh enriched fat compared to the control group was higher in the enriched group and showed an average difference of 1.681 ($P > 0.05$). The weekly growth rate of tumor volume in the non-enriched fat group was higher compared to the control group and showed an average difference of 1.423 ($P > 0.05$). A comparison of tumor growth rate between the two groups showed a higher growth rate in the fat-enriched group, albeit not statistically significant.

The results indicate that this type of experimental model is an effective measurable method that will allow us in the future to investigate the effect of autologous fat transfer on breast cancer.

DISCUSSION

The aim of this study was to establish an efficient experimental model, using MRI scans, to compare different breast tumor study groups after fat grafting.

MRI is considered a beneficial modality for the rapid and precise volume analysis of breast tissue and carcinoma of breast, mainly after autologous fat grafting, and is currently an excellent method for imaging structural changes due to fat grafting, enabling a cost-benefit and accurate follow-up [14]. Moreover, breast MRI is a capable imaging tool for the early detection of autologous fat necrosis, fat survival and other complications. It has the capacity to distinguish malignant from benign lesion in the breast, and should be performed as early as possible after the fat grafting procedure [15].

The ability to detect changes during a longitudinal study [16], such as qualitative measurements, has been shown in long-term follow-up of gluteal fat grafting [17], vocal cord fat grafting [18] and facial fat grafting [19], emphasizing the potential important role of MRI in long-term breast volume assessments after fat grafting.

Although not statistically significant, our study yielded important findings regarding tumor growth rate. In summarizing the MRI measurements, we noticed a substantial difference in the weekly average tumor growth rate among the AdMSC-enriched fat and non-enriched fat groups as compared to the control group.

The ability to quantify the tumor growth rate after enriched and non-enriched fat grafting by using MRI scans in SCID mice allowed us to assess each group individually and then compare the rate with the other groups. In other words, we were able to assess the influence of enriched and non-enriched fat grafting on the volume and growth rate of breast tumors.

LIMITATIONS

The lack of statistical significance may be due to our small sample size. A larger study might reveal significant outcomes. However, since we did not aim to assess the influence of fat grafting, but rather the validity of the model, this does not detract from the importance of our findings.

IMPLICATIONS AND FUTURE RESEARCH

An MRI modal study with a significant number of mice is needed to examine the influence of grafted fat or grafted fat enriched with mesenchymal progenitor cells on breast cancer recurrence.

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