

Fat Graft Safety after Oncologic Surgery: Addressing the Contradiction between In Vitro and Clinical Studies

Hakan Orbay, M.D., Ph.D.
Katharine M. Hinchcliff,
M.D.
Heath J. Charvet, M.D.
David E. Sahar, M.D.

Sacramento, Calif.



PATIENT
SAFETY



Background: The authors investigate the in vitro and in vivo interaction of human breast cancer cells and human adipose-derived stem cells to address the controversy on the safety of postmastectomy fat grafting.

Methods: The authors co-cultured human adipose-derived stem cells and MDA-MB-231 breast cancer cells in an in vitro cell migration assay to examine the migration of breast cancer cells. In the in vivo arm, the authors injected breast cancer cells (group I), human breast cancer cells plus human adipose-derived stem cells (group II), human breast cancer cells plus human fat graft (group III), and human breast cancer cells plus human fat graft plus human adipose-derived stem cells (group IV) to the mammary fat pads of female nude mice ($n = 20$). The authors examined the tumors, livers, and lungs histologically after 2 weeks.

Results: Migration of breast cancer cells increased significantly when co-cultured with adipose-derived stem cells ($p < 0.05$). The tumor growth rate in group IV was significantly higher than in groups I and II ($p < 0.05$). The tumor growth rate in group III was also higher than in groups I and II, but this difference was not statistically significant ($p > 0.05$). Histologically, there was no liver/lung metastasis at the end of 2 weeks. The vascular density in the tumors from group IV was significantly higher than in other groups ($p < 0.01$).

Conclusion: The injection of breast cancer cells, fat graft, and adipose-derived stem cells together increases breast cancer xenograft growth rates significantly. (*Plast. Reconstr. Surg.* 142: 1489, 2018.)

Excision of breast cancer often leads to disfigurement and asymmetry of the breast that requires reconstruction. A recent addition to the field of breast reconstruction is fat grafting.¹ Fat grafting was once banned by the American Society of Plastic Surgeons because of raised concerns about its interference with the detection of breast cancer.² However, since the early 2000s, fat grafting has been rediscovered and reassessed.^{3,4} As supportive evidence for the

Disclosure: *The authors have no financial interest to declare in relation to the content of this article.*

Supplemental digital content is available for this article. Direct URL citations appear in the text; simply type the URL address into any Web browser to access this content. Clickable links to the material are provided in the HTML text of this article on the *Journal's* website (www.PRSJournal.com).

By reading this article, you are entitled to claim one (1) hour of Category 2 Patient Safety Credit. ASPS members can claim this credit by logging in to PlasticSurgery.org Dashboard, clicking “Submit CME,” and completing the form.

A “Hot Topic Video” by Editor-in-Chief Rod J. Rohrich, M.D., accompanies this article. Go to PRSJJournal.com and click on “Plastic Surgery Hot Topics” in the “Digital Media” tab to watch.

From the Department of Surgery, Division of Plastic Surgery, University of California, Davis Medical Center.

Received for publication March 2, 2017; accepted April 19, 2018.

Presented at the 61st Annual Meeting of the Plastic Surgery Research Council, in New York, New York, May 19 through 22, 2016; and the 14th Annual International Conference of the International Federation for Adipose Therapeutics and Science, in San Diego, California, November 17 through 20, 2016.

Copyright © 2018 by the American Society of Plastic Surgeons

DOI: 10.1097/PRS.0000000000004992

safety of fat grafting increased, the position of the American Society of Plastic Surgeons has also evolved. In 2009, the American Society of Plastic Surgeons Fat Graft Task Force lifted the ban on autologous fat grafting and released new recommendations on the safety and efficacy of postmastectomy fat grafting.⁵ The Task Force reported no interference with breast cancer surveillance after fat grafting to the breast; however, it did not discuss whether fat grafting modifies the risk of breast cancer recurrence in postresection patients.⁵

Since that time, the incidence of fat grafting as an adjunct to breast reconstruction has increased significantly. Relatively low fat graft retention rates, estimated between 20 and 75 percent,^{6,7} have discouraged surgeons from using fat grafting as a primary method of reconstruction, and can require patients to undergo multiple treatments to achieve the desired result. However, the modification of enriching fat grafts, or co-injecting a preexpanded adipose-derived stem cell population, has shown great promise in increasing fat graft retention.^{8,9}

Adipose-derived stem cells are known to secrete growth factors and cytokines that promote tissue regeneration and revascularization, processes important for fat survival but also critical to cancer growth and metastasis (Fig. 1).¹⁰⁻¹³ This discovery brought in a major controversy on whether fat grafting will increase the risk of tumor recurrence in postmastectomy patients. The most striking aspect of this controversy is the opposing results obtained from basic scientific and clinical studies. Several groups have repeatedly documented that adipose-derived stem cells increase the growth and migration of breast cancer cells under experimental conditions.^{14,15} Interestingly, clinical studies failed to document any increase in breast cancer recurrence caused by postmastectomy fat grafting.¹⁶⁻¹⁸ Cases of breast cancer recurrence that may be linked to fat grafting have been reported; however, these are sporadic and the number of patients too few for a definitive conclusion.^{19,20} In this study, we address this controversy by examining the *in vivo* and *in vitro* interactions of human adipose-derived stem cells and breast cancer cells using a clinically relevant experimental design.

MATERIALS AND METHODS

The human and animal experiments were approved by the Institutional Review Board

(approval no. 254494-9) and Institutional Animal Care and use Committee (approval no. 18296).

In Vitro Migration Assay

In the *in vitro* arm of the study, we examined the interaction of breast cancer cells and human adipose-derived stem cells using a migration assay chamber (Cell Biolabs, Inc., San Diego, Calif.). We obtained adipose tissue from patients ($n = 3$) by means of liposuction and harvested human adipose-derived stem cells from these samples by means of enzymatic digestion using our previously published technique.¹⁴ The migration assay was performed as instructed by the manufacturer. In study groups, we seeded the lower chamber with human adipose-derived stem cells from passages III to V at a density of 1.0×10^6 /ml. In control groups, the lower chamber was filled with either cell culture medium (Dulbecco's Modified Eagle Medium, 10% fetal bovine serum, and 1% antibiotic/antimycotic solution or phosphate-buffered saline; all from GE Healthcare Bio-Sciences, Pittsburgh, Pa.). All of the groups were set up in triplicate. We quantified the number of migrated breast cancer cells in each group using a fluorescent plate reader (Molecular Devices, Sunnyvale, Calif.) and expressed the results in relative fluorescence units.

In Vivo Study

For the *in vivo* arm of the study, we harvested fat tissue from a single donor by means of liposuction. We divided the fat tissue into two halves and isolated stromal vascular fraction cells from the first half by means of enzymatic digestion as described previously.^{14,21} The second half was processed by means of the Coleman technique for fat graft.³ We labeled the stromal vascular fraction cells with 3,3'-dioctadecyloxycarbocyanine, perchlorate (DiO) for *in vivo* tracking purposes. This mixture of stromal vascular fraction, MDA-MB-231/red fluorescent protein-positive human breast cancer cells, and fat graft was then injected into the bilateral fourth mammary fat pads of female nude mice using 18-gauge needle tips ($n = 20$) as shown in Table 1. The number of cells in each group was 8×10^5 breast cancer cells and 1.8×10^5 stromal vascular fraction to each mammary fat pad. In this study, we used a lower number of breast cancer cells for the *in vivo* arm to obtain a more clinically relevant experimental model. We followed tumor growth with digital caliper measurements every other day and calculated the tumor volume using the formula ($\text{length} \times \text{width}^2$)/2. The animals were killed 2 weeks after

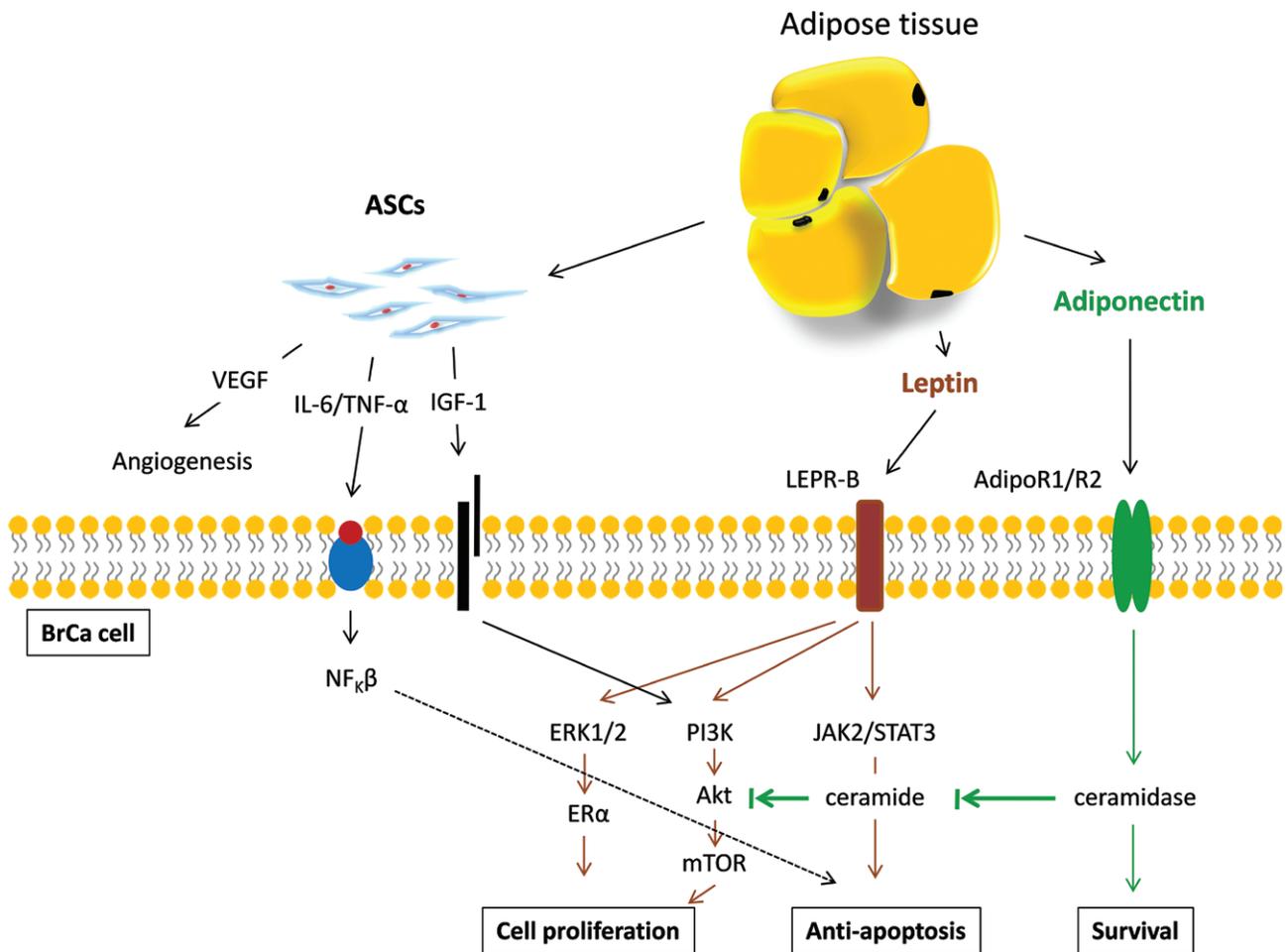


Fig. 1. Main pathways of interaction between adipose tissue and breast cancer cells. *ASCs*, adipose-derived stem cells; *VEGF*, vascular endothelial growth factor; *IL-6*, interleukin-6; *TNF-α*, tumor necrosis factor-α; *IGF-1*, insulin like growth factor-1; *LEPR-B*, leptin receptor-B; *AdipoR1/R2*, adiponectin receptor 1/2; *NFκB*, nuclear factor kappa-light-chain-enhancer of activated B cells; *ERK1/2*, mitogen-activated protein kinase 3/1; *ERα*, estrogen receptor alpha; *PI3K*, phosphatidylinositol-4,5-bisphosphate 3-kinase; *Akt*, protein kinase B; *mTOR*, mechanistic target of rapamycin; *JAK2/STAT3*, Janus kinase 2/signal transducer and activator of transcription 3. (Adapted from Park J, Euhus DM, Scherer PE. Paracrine and endocrine effects of adipose tissue on cancer development and progression. *Endocr Rev.* 2011;32:550–570; and van Kruijsdijk RC, van der Wall E, Visseren FL. Obesity and cancer: The role of dysfunctional adipose tissue. *Cancer Epidemiol Biomarkers Prev.* 2009;18:2569–2578.)

Table 1. Study Groups

Group	Injected Mixture	Injection Volume (μl)	
		Cell Suspension	Fat Graft
I	BrCa only	150	—
II	BrCa + hASCs	150	—
III	BrCa + fat graft	75	75
IV	BrCa + hASCs + fat graft	75	75

BrCa, breast cancer cells; hASCs, human adipose-derived stem cells.

inoculation, and the tumors, lungs, and livers were removed for histologic examination.

Histology

The excised tumors and organs were embedded in optimal cutting temperature compound

(VWR International, Radnor, Pa.) and frozen in cold acetone (EMD Millipore, Billerica, Mass.). The frozen blocks were cut into 5-μm sections using a cryostat and stained using hematoxylin and eosin and immunofluorescence.

For hematoxylin and eosin staining of the breast cancer tumors, we randomly selected one tumor from every animal in each group (*n* = 5) and obtained five sections from each tumor. We used these sections to evaluate the general composition of the tumors and also to measure the percentage volume of fat tissue in each tumor using ImageJ software (National Institutes of Health, Bethesda, Md.).²² We subtracted the percentage volume of fat tissue in each group from the total volume of breast cancer tumors that we obtained with digital caliper

measurements. The rationale for doing this was to eliminate the contribution of remaining fat graft volume to the total tumor volume in groups III and IV (i.e., a false increase in breast cancer tumor volume) so that we could make a comparison of only breast cancer tumor volumes. For hematoxylin and eosin staining of livers and lungs, we randomly selected three animals from each group and obtained 12 sections from the livers and lungs of these animals. We used these sections to detect organ micrometastasis from the primary breast cancer tumor.

We examined the sections from groups II and IV under fluorescence microscopy before immunofluorescence staining to detect any DiO-positive stromal vascular fraction cells. Afterward, we performed von Willebrand factor immunofluorescence staining to detect the average vessel density in tumors. In every animal, we randomly selected either the right or the left breast cancer xenograft and obtained 12 sections from that tumor, for a total of 60 sections per group. We incubated the tissue sections with a rabbit anti-von Willebrand factor primary antibody (Abcam, Inc., Cambridge, Mass.) overnight at 4°C. We used anti-rabbit fluorescence isothiocyanate (Abcam) as a secondary antibody. The nuclei were counterstained with 4',6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, Calif.), and the images were captured under a fluorescence microscope (Zeiss AxioObserver.Z1; Carl Zeiss, Oberkochen, Germany). The number of vessels in each section was counted manually.

In addition to hematoxylin and eosin staining of livers and lungs, we performed anti-red fluorescent protein immunofluorescence staining to detect breast cancer micrometastasis in these organs. We randomly selected three animals from each group and obtained 12 sections from the livers and lungs of these animals. We incubated the tissue sections with a rabbit anti-red fluorescent protein antibody (Thermo Scientific, Rockford, Ill.). Counterstaining of the nuclei and imaging was performed as described above.

Statistical Analysis

All results were compared using the one-way analysis of variance test and, if necessary, the Tukey test. A value of $p < 0.05$ was considered significant.

RESULTS

In Vitro Migration Assay

Two of three human adipose-derived stem cell populations increased the migration of breast cancer cells significantly ($p < 0.05$) (Fig. 2). The third human adipose-derived stem cell line also

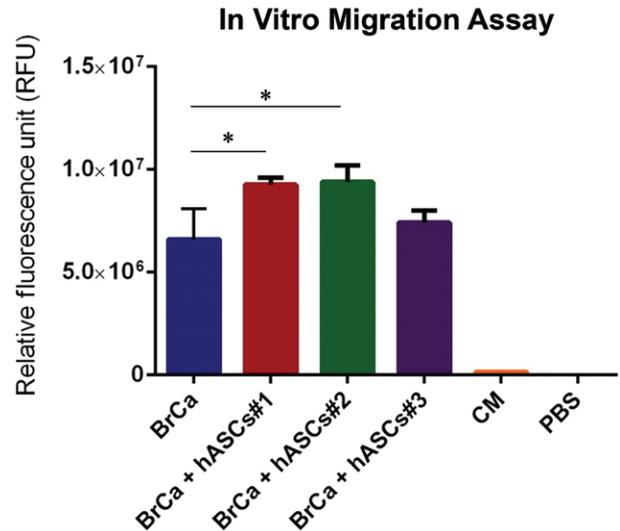


Fig. 2. Graph summarizing the results of in vitro migration assay. Human adipose-derived stem cell populations 1 and 2 significantly increased the migration of breast cancer cells. * $p < 0.05$. BrCa, breast cancer cells; hASCs, human adipose-derived stem cells; CM, culture medium; PBS, phosphate-buffered saline.

increased the migration of breast cancer cells but not to a statistically significant degree ($p > 0.05$). The average fluorescence from the wells with only breast cancer cells was $6.5 \times 10^6 \pm 1.4 \times 10^6$ relative fluorescence units, whereas the average fluorescence from the wells with human adipose-derived stem cells and breast cancer co-cultures were $9.2 \times 10^6 \pm 3.2 \times 10^5$ ($p < 0.05$), $9.3 \times 10^6 \pm 8.1 \times 10^5$ ($p < 0.05$), and $7.4 \times 10^6 \pm 5.7 \times 10^5$ ($p > 0.05$) relative fluorescence units for human adipose-derived stem cell lines 1, 2, and 3, respectively. The fluorescence from control wells containing only culture medium and phosphate-buffered saline were negligible (Fig. 2).

In Vivo Study

All of the animals survived surgery and eventually developed tumors at the injection sites. Before subtraction of the fat graft volume, the average breast cancer tumor volumes on day 15 were 341.2 ± 155.1 , 365.4 ± 148.2 , 748.7 ± 378.6 , and 1121.6 ± 667.3 mm³ in groups I, II, III, and IV, respectively. (See Figure, Supplemental Digital Content 1, which shows the graph with the tumor volumes measured initially and before subtracting the percentage volume of fat tissue in each tumor, <http://links.lww.com/PRS/D97>.) The tumor volumes after adjustment were 327.2 ± 148.8 , 344.2 ± 151.9 , 677.3 ± 145.1 , and 1024.62 ± 481.6 mm³ in groups I, II, III, and IV, respectively (Fig. 3). The tumors in group IV (breast cancer cells plus adipose-derived stem cells plus fat graft) were larger

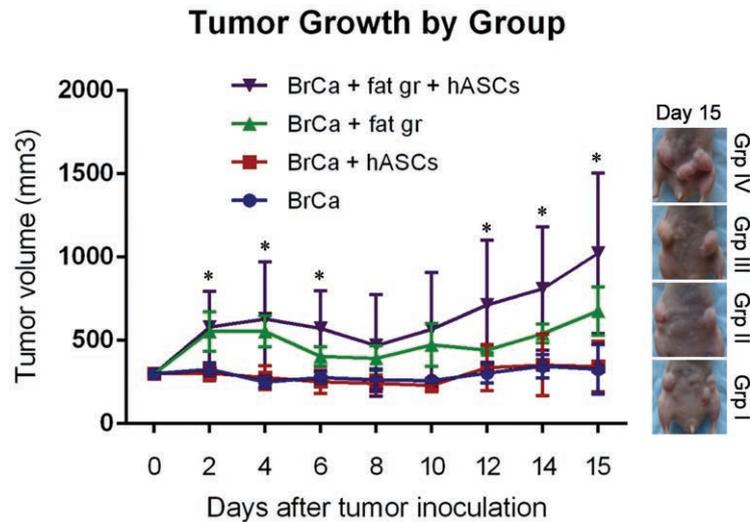


Fig. 3. Graph showing the tumor growth rates in the study groups after subtraction of the volume of fat tissue. Group IV injected with breast cancer cells plus adipose-derived stem cells plus fat graft showed the highest rate of tumor growth. The difference between group IV and groups I and II was significant at almost all time points as marked by asterisks. The tumors in group III injected with breast cancer cells plus fat graft also grew faster than the tumors in groups I and II, but this difference did not achieve statistical significance. Injection of human adipose-derived stem cells with breast cancer cells (group II) did not affect the tumor growth rates compared with breast cancer cells only (group I). * $p < 0.05$.

than the tumors in groups I (breast cancer cells only), II (breast cancer plus adipose-derived stem cells), and III (breast cancer cells plus fat graft) at almost all time points ($p < 0.05$). There was no difference between the tumor volumes in group III versus groups I and II ($p > 0.05$) except on day 2 after injection (Fig. 3). The adjustment of the tumor volumes by subtracting the fat tissue volume did not change our statistical results.

Histology

Hematoxylin and eosin staining of the tumors revealed that almost all of the fat tissue injected in groups III and IV was replaced by breast cancer tissue (Fig. 4, left). The percentage fat volumes within the tumors in groups I, II, III, and IV were 4.1 ± 2.7 , 5.8 ± 3.5 , 11.4 ± 6.7 , and 11.9 ± 6.4 percent, respectively. There was no significant difference across the groups ($p < 0.05$) (Fig. 4, right). Because the adjustment of the tumor volumes by subtracting the percentage fat volume from the total tumor volume did not alter our statistical results, we concluded that the injected fat tissue in groups III and IV did not contribute significantly to the final tumor volume.

The vascular density (vessels per high-power field) in the tumors from group IV (20.3 ± 3.1)

was significantly higher in comparison with tumors from groups I (12.3 ± 1.7), II (12.7 ± 2.3), and III (18.8 ± 3.7) ($p < 0.01$) (Fig. 5). We failed to observe any DiO-positive stromal vascular fraction cells remaining within the tumors after 2 weeks (Fig. 6). This suggests that the injected stromal vascular fraction cells did not survive in vivo.

Hematoxylin and eosin staining of livers and lungs did not reveal any micrometastasis in these organs (Fig. 7). These results were confirmed with red fluorescent protein immunofluorescence staining. We could not detect any red fluorescent protein-positive breast cancer cells in the livers and lungs of the animals (Fig. 8).

Summary of Findings

In our in vitro migration assay, we found that adipose-derived stem cells increased the migration of breast cancer cells. The in vivo arm of this study demonstrated that co-injection of adipose-derived stem cells and breast cancer cells and of fat graft with breast cancer cells did not significantly increase tumor growth. However, we did find that, when adipose-derived stem cells and fat graft are co-injected with breast cancer cells (analogous to an enriched fat graft), tumor growth increased significantly.

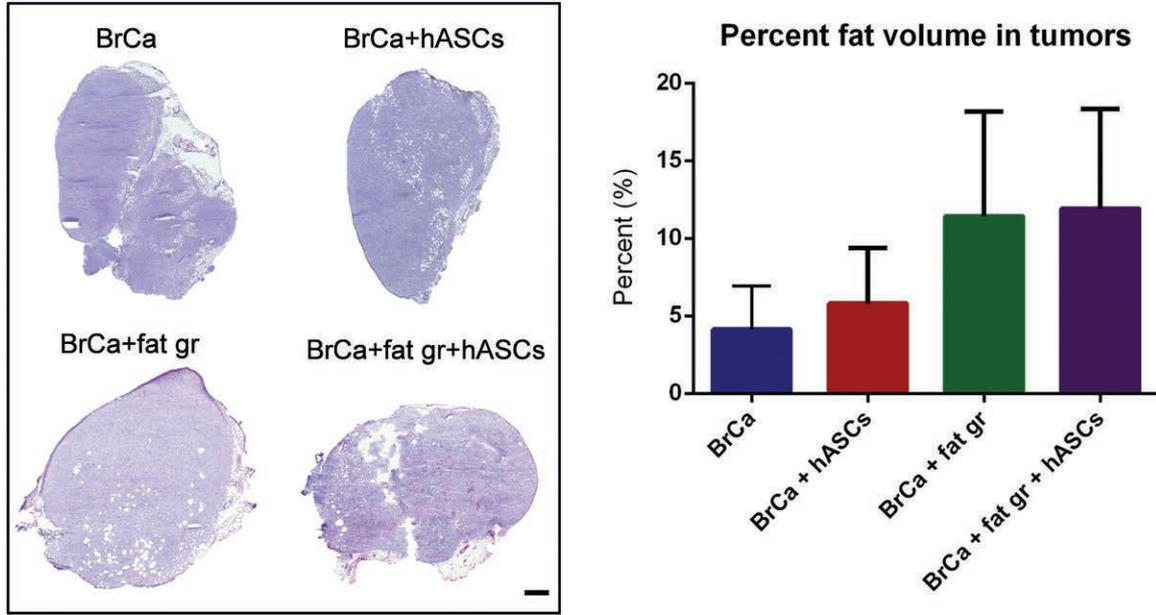


Fig. 4. (Left) Representative hematoxylin and eosin–stained sections of the breast cancer xenografts from each group. The fat tissue injected in groups III and IV was largely replaced by breast cancer tissue, but there were still varying amounts of fat tissue within the tumors. *Microbar* = 500 μ m. (Right) The largest amount of remaining fat tissue was in group IV in terms of percentage volume, but the difference between the groups was not significant.

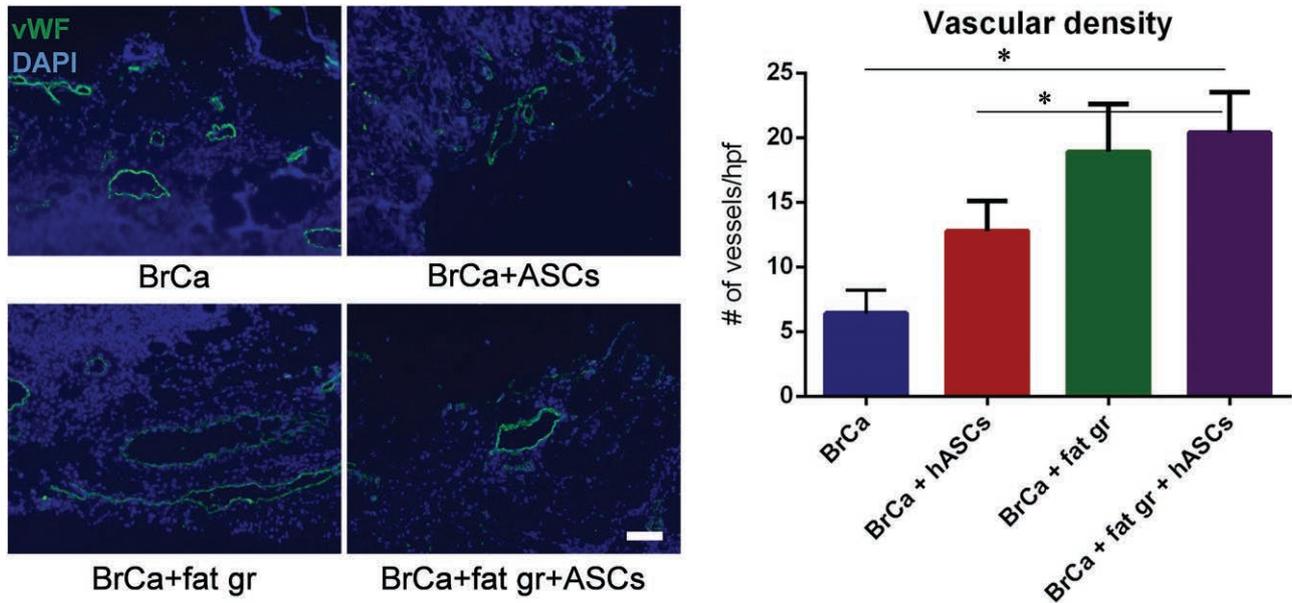


Fig. 5. (Left) Von Willebrand factor staining for vessel count. The nuclei are seen in *blue*, whereas the von Willebrand factor–positive vascular endothelium is seen in *green*. (Right) The average number of vessels per high-power field was highest in group IV. * $p < 0.05$. *Microbar* = 100 μ m.

DISCUSSION

Experimental Design

In this study, we aimed to inject equal doses of adipose-derived stem cells (1.8×10^5) into all the animals in study groups II and IV to standardize

our experimental methods. However, because the fat grafts that we injected into animals in groups III and IV also contained adipose-derived stem cells, we have inevitably injected more human adipose-derived stem cells into mice in these groups. In contrast, 1 ml of lipoaspirate yields an average of

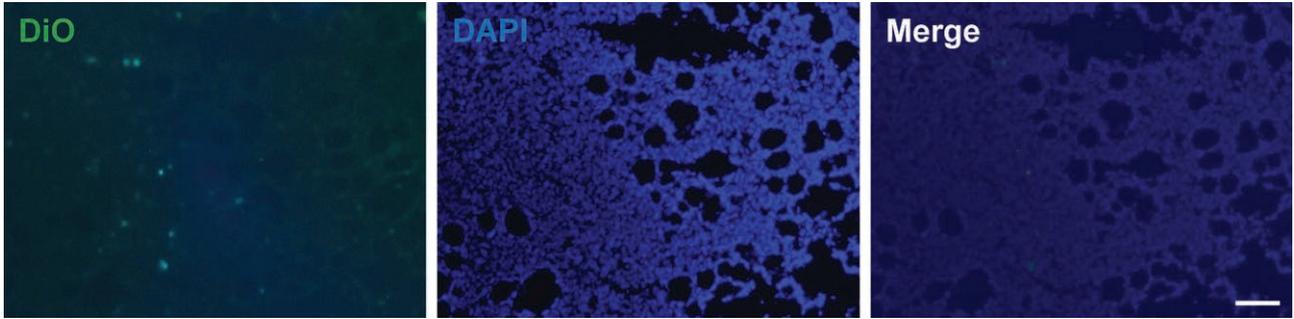


Fig. 6. There were no DiO-positive human adipose-derived stem cells within the xenografts after 2 weeks, suggesting that the injected human adipose-derived stem cells did not survive. *Microbar* = 1000 μ m. *DAPI*, 4',6-diamidino-2-phenylindole.

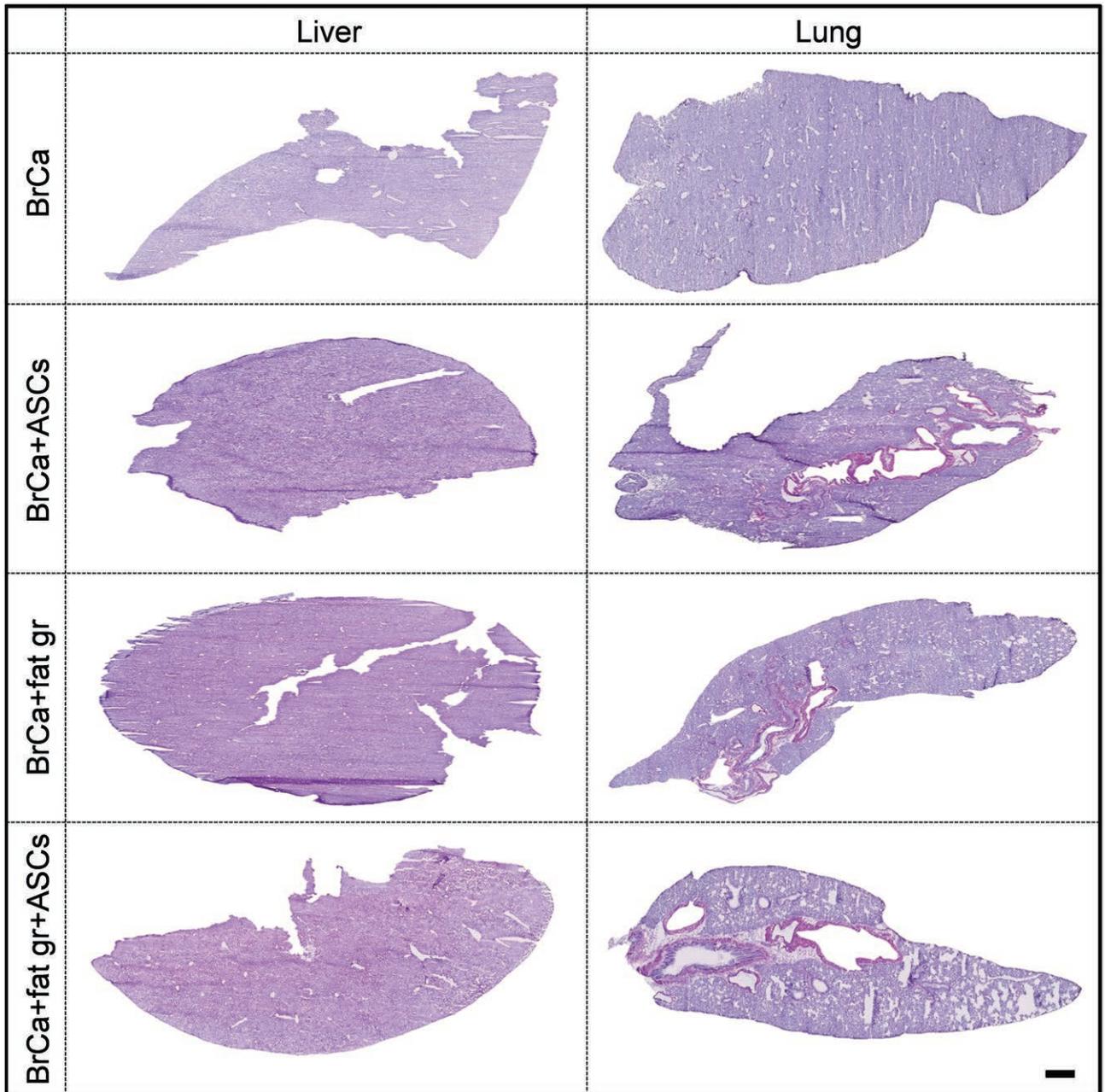


Fig. 7. Representative images of hematoxylin and eosin–stained sections from livers and lungs of mice in study groups. There was no visible micrometastasis in either of these organs. *Microbar* = 1000 μ m. *BrCa*, breast cancer cells; *ASCs*, adipose-derived stem cells.

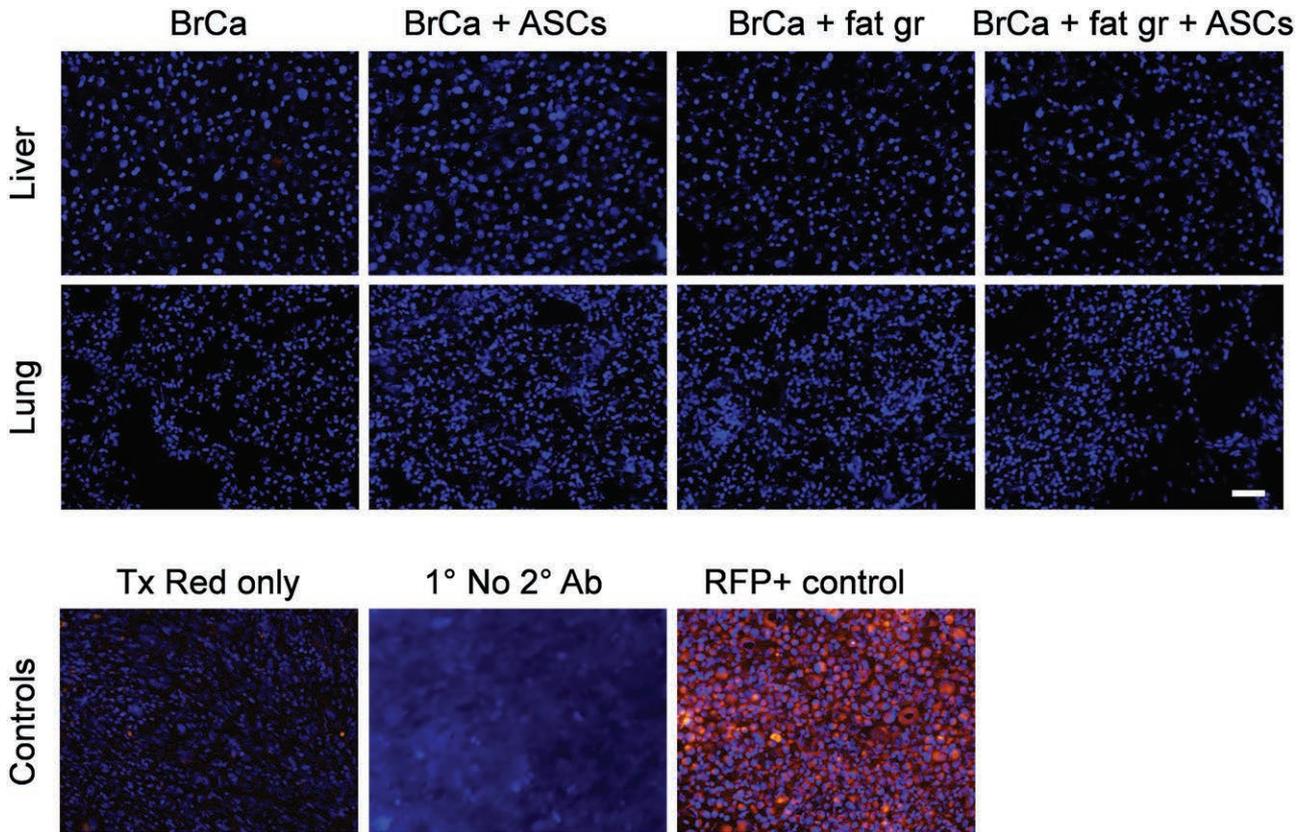


Fig. 8. Results of red fluorescent protein staining. The breast cancer cells stained positive for red fluorescent protein (below, right); however, there was no positivity in the lung or liver sections. *Microbar* = 500 μ m. *BrCa*, breast cancer cells; *ASCs*, adipose-derived stem cells; *Tx Red*, Texas Red; *Ab*, antibody; *RFP+*, red fluorescent protein–positive.

3.75×10^5 cells, and we injected 0.075 ml of fat per injection site; therefore, there were approximately 2.8×10^4 adipose-derived stem cells in the fat grafts, which is quite negligible compared to the number of adipose-derived stem cells injected in groups II and IV (1.8×10^5). We therefore hypothesized that it was the interactions between the fat grafts and adipose-derived stem cells that resulted in significantly higher breast cancer growth rates in the adipose-derived stem cell plus fat graft group, as opposed to the raw human adipose-derived stem cell numbers. In addition, if raw numbers of human adipose-derived stem cells were the main determining factor for tumor growth, we would expect group II to have grown larger tumors than group III, which we did not observe.

With respect to fat quantification within tumors, the reader will note that we did not use perilipin or any other specific immunologic staining to quantify the fat tissue within the tumors. Instead, we quantified the amount of fat tissue based on hematoxylin and eosin–stained slides. Our rationale for this method of quantification was to avoid the error introduced by background

staining that is frequently encountered in immunologic staining methods as we have also observed in our previous experiments. Even though it is a simpler staining method compared with immunologic staining, we found hematoxylin and eosin staining to be more accurate in certain cases, given that a careful evaluation and observation is performed under the microscope.

Results

Late recurrence of breast cancer is usually attributable to dormant cancer cells.²³ Much of the controversy in the literature regarding the interaction of human adipose-derived stem cells with dormant breast cancer cells stems from opposing results of in vitro and in vivo/clinical studies as observed in our study. Human adipose-derived stem cells increased the in vitro migration of breast cancer cells, but injection of human adipose-derived stem cells with breast cancer cells to the mammary fat pads of mice did not increase the tumor growth rate. There might be several reasons for this discrepancy stemming from the in vitro and in vivo behavior of human adipose-derived stem cells. Several articles

reported that injected mesenchymal stem cells do not survive in vivo because of the lack of an immediate vascular supply after transplantation.^{24,25} The cell growth medium effectively delivers the nutrients to the cells in the in vitro cultures, but the access of the cells to the nutrients in vivo relies solely on neovascularization after transplantation. A delay in neovascularization unquestionably leads to cell death and decreases the biological effectiveness of the injected cells. Therefore, adipose-derived stem cells in our study might not have survived long enough to exhibit their stimulatory biological effects on breast cancer cells, except in the breast cancer cell plus adipose-derived stem cell plus fat graft group, where the adipose-derived stem cells may have lasted longer because of trophic support from being in their native environment. In addition, breast cancer cells are fast-growing cells and potentially outcompeted the adipose-derived stem cells in terms of access to locally available nutrients and blood supply, thereby accelerating the death of adipose-derived stem cells in vivo. These hypotheses were supported by the lack of DiO-positive adipose-derived stem cells in the histologic sections of breast cancer xenografts at the end of 2 weeks.

The complexity of the in vivo environment is another potential reason for the differential findings between in vivo and in vitro studies. Even though adipose-derived stem cells increased the migration of breast cancer cells in vitro, the in vivo environment is much more complex, and several other cues come into play that may interrupt the interaction between breast cancer cells and adipose-derived stem cells.²³ Adipose-derived stem cells secrete multiple important growth factors, cytokines, chemokines, and inflammatory biomarkers linked to cancer development and progression.²⁶ Adipose tissue is also a highly active endocrine organ and can influence distant tumor sites by means of endocrine function and soluble factors.²⁷ These soluble factors, such as leptin and estrogen, facilitate interactions between stromal cells and tumor cells, creating a specific peritumoral microenvironment (Fig. 1).^{27,28} When adipose-derived stem cells are transferred to a new location in their natural environment (i.e., mixed with fat grafts such as in group IV in this study), the preserved interaction between the fat tissue and adipose-derived stem cells may potentiate the effects of both tissues, thereby explaining the increased breast cancer xenograft growth rates that we observed in group IV (i.e., breast cancer cells plus adipose-derived stem cells plus fat graft). Another potential hypothesis could be that the absolute number of adipose-derived

stem cells starting out in a sample is the main factor impacting tumor growth. However, we believe this to be relatively less likely, as we would expect the group with breast cancer and adipose-derived stem cells to grow relatively more than the breast cancer cell plus fat graft group, which we did not see.

Another confounding factor in the literature is that the majority of the published experimental studies use immortalized cancer cell lines, which are aggressive and designed to give rise to large tumors in a very short time. This experimental model fails to approximate the crucial aspects of tumor heterogeneity, tumor dormancy, and reactivation of occult tumor cells.²³ The experimental models using immortalized breast cancer cells bear relevance only to rapidly growing, high-grade breast cancer tumors, where reconstructive surgery likely would not be a consideration.²³ The importance of tumor heterogeneity was also emphasized by studies by Donnenberg et al.^{29,30} In these studies, despite the fact that all breast cancer cells were phenotypically the same (CD90⁺), human adipose-derived stem cells failed to augment the tumorigenicity of dormant breast cancer cells, whereas they markedly enhanced tumorigenesis mediated by the active cells. Based on this finding, the authors claimed that the transition between dormant and active states requires genetic reprogramming and not merely the presence of signals such as those provided by cytokines and growth factors secreted by adipose-derived stem cells. Therefore, they argued that the introduction of adipose-derived stem cells to the site of a tumor bed would be noncontributory to local recurrence.

We did not observe any increase in the internal organ micrometastasis with the injection of fat grafts or enriched fat grafts. However, in our pilot study, we allowed the mice to survive for 3 weeks instead of 2 weeks and observed several internal organ metastases in the livers of mice. The number of liver micrometastases in group IV, injected with enriched fat grafts, was significantly higher compared with other groups. (See **Figure, Supplemental Digital Content 2**, which shows the micrometastases in the livers of the mice that survived for 3 weeks in our pilot study. *Dashed circles* point to the metastases. *Microbar* = 50 μ m. The number of micrometastases was significantly higher in group IV compared with other groups. * $p < 0.05$. *BrCa*, breast cancer cells; *hASCs*, human adipose-derived stem cells, <http://links.lww.com/PRS/D98>.) It is a well-known fact that organ metastasis of malignant tumors is a time-dependent event. The more

advanced the tumor, the higher the risk of internal organ metastasis; this may explain the lack of internal organ metastases at the 2-week mark in our study. We allowed the animals to survive for just 2 weeks in the current study because the tumor burden that we observed in the pilot study was concerning from an ethical standpoint. Based on these results, we hypothesize that enriched fat grafting increases not only breast cancer growth but also internal organ metastases in a time-dependent fashion. Importantly, this hypothesis is relatively weakened by the limitations of our model. Despite our best efforts, animal models are not perfect representations of human disease. In our *in vivo* study, we chose an immunocompromised model to avoid rejection of human fat grafts. Inoculation of a highly aggressive human breast cancer cell line in an immunocompromised murine model may not accurately reflect the complex tumor-host interactions in the majority of breast cancer patients.

The existing clinical data convincingly documented the lack of increased breast cancer recurrence rates after postmastectomy fat grafting.³¹ Petit et al. in 2011 published a multicenter analysis of 513 patients who underwent fat grafting and reported that the local and distant recurrence rates were comparable with the patients who did not undergo postmastectomy fat grafting.¹⁷ The same group analyzed 321 consecutive patients against a 1:2 matched cohort with similar characteristics and found no difference in recurrence rates.¹⁶ However, when analysis was limited to a subset of 37 patients with intraepithelial neoplasms, the local recurrence rate increased significantly to 10.8 percent versus none in the cohort control.¹⁶ The initial findings prompted the team to perform a matched cohort study of 59 patients with intraepithelial neoplasms compared to 118 matched control patients with intraepithelial neoplasms who did not receive fat grafts. This study revealed an 18 percent 5-year cumulative risk of local recurrence in the breast fat grafting group compared to 3 percent in the control cohort ($p = 0.02$).¹⁸ Intraepithelial neoplasms being an exception, following clinical articles again failed to detect any increase in breast cancer recurrence rates after postmastectomy fat grafting.^{32,33} In a previously published literature review, we compiled 16 clinical studies including 2100 patients and found that the overall rate of local breast cancer recurrence after fat grafting was 2.2 percent.³⁴ These results were again comparable to the figures reported in patients who did not undergo postmastectomy fat grafting.^{35,36} Our *in vivo* results parallel results of clinical studies, with both

demonstrating that nonenriched fat grafts do not appear to increase tumor growth in the oncologic patient. Thus, our data help clarify the ongoing discrepancy between the basic scientific and clinical studies showing that fat graft alone does not appear to impact tumor growth.

Fat grafting is a useful tool for breast reconstruction and augmentation, and adipose-derived stem cells increase the viability of injected fat grafts.^{37,38} However, it is important to note that the use of enriched fat grafting in our model did significantly increase breast cancer growth, suggesting that enriched autologous fat grafting in the oncologic patient could potentially increase recurrence rates and growth of any residual breast cancer cells. Therefore, despite the temptation because of improved fat graft retention, we strongly recommend caution and close surveillance when using enriched fat grafting for oncologic breast reconstruction until more robust clinical safety data are available. In addition, we have changed our aesthetic practice such that we do not offer enriched fat grafting to augmentation patients with a family history of breast cancer genes such as *BRCA1* and *BRCA2*.

CONCLUSIONS

Fat grafting alone does not increase the growth of breast cancer xenografts significantly. Combined with the clinical data in the literature, our results demonstrate the safety of fat grafting for postmastectomy and oncologic breast reconstruction. However, co-injection of fat grafts enriched with adipose-derived stem cells should be used with caution, at least until cancer remission can be firmly established and higher level clinical data are available.

Hakan Orbay, M.D., Ph.D.

Division of Plastic Surgery

University of California, Davis Medical Center

4625 2nd Avenue, Room 3001

Sacramento, Calif. 95817

horbay@ucdavis.edu

REFERENCES

1. Spear SL, Coles CN, Leung BK, Gitlin M, Parekh M, Macarios D. The safety, effectiveness, and efficiency of autologous fat grafting in breast surgery. *Plast Reconstr Surg Glob Open* 2016;4:e827.
2. Report on autologous fat transplantation. ASPRS Ad-Hoc Committee on New Procedures, September 30, 1987. *Plast Surg Nurs*. 1987;7:140-141.
3. Coleman SR. Hand rejuvenation with structural fat grafting. *Plast Reconstr Surg*. 2002;110:1731-1744; discussion 1745-1747.

4. American Society of Plastic Surgeons. 2012 plastic surgery statistics. Available at: <http://www.plasticsurgery.org/news-and-resources/2012-plastic-surgery-statistics.html>. Accessed October 8, 2013.
5. Gutowski KA; ASPS Fat Graft Task Force. Current applications and safety of autologous fat grafts: A report of the ASPS Fat Graft Task Force. *Plast Reconstr Surg*. 2009;124:272–280.
6. Coleman SR. Structural fat grafts: The ideal filler? *Clin Plast Surg*. 2001;28:111–119.
7. Khouri R, Del Vecchio D. Breast reconstruction and augmentation using pre-expansion and autologous fat transplantation. *Clin Plast Surg*. 2009;36:269–280, viii.
8. Kølbe SF, Fischer-Nielsen A, Mathiasen AB, et al. Enrichment of autologous fat grafts with ex-vivo expanded adipose tissue-derived stem cells for graft survival: A randomised placebo-controlled trial. *Lancet* 2013;382:1113–1120.
9. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: Implications for cell-based therapies. *Tissue Eng*. 2001;7:211–228.
10. Bertolini F, Petit JY, Kolonin MG. Stem cells from adipose tissue and breast cancer: Hype, risks and hope. *Br J Cancer* 2015;112:419–423.
11. Schweizer R, Tsuji W, Gorantla VS, Marra KG, Rubin JP, Plock JA. The role of adipose-derived stem cells in breast cancer progression and metastasis. *Stem Cells Int*. 2015;2015:120949.
12. Park J, Euhus DM, Scherer PE. Paracrine and endocrine effects of adipose tissue on cancer development and progression. *Endocr Rev*. 2011;32:550–570.
13. van Kruijsdijk RC, van der Wall E, Visseren FL. Obesity and cancer: The role of dysfunctional adipose tissue. *Cancer Epidemiol Biomarkers Prev*. 2009;18:2569–2578.
14. Charvet HJ, Orbay H, Harrison L, Devi K, Sahar DE. In vitro effects of adipose-derived stem cells on breast cancer cells harvested from the same patient. *Ann Plast Surg*. 2016;76(Suppl 3):S241–S245.
15. Chandler EM, Seo BR, Califano JP, et al. Implanted adipose progenitor cells as physicochemical regulators of breast cancer. *Proc Natl Acad Sci USA* 2012;109:9786–9791.
16. Petit JY, Botteri E, Lohsiriwat V, et al. Locoregional recurrence risk after lipofilling in breast cancer patients. *Ann Oncol*. 2012;23:582–588.
17. Petit JY, Lohsiriwat V, Clough KB, et al. The oncologic outcome and immediate surgical complications of lipofilling in breast cancer patients: A multicenter study. Milan-Paris-Lyon experience of 646 lipofilling procedures. *Plast Reconstr Surg*. 2011;128:341–346.
18. Petit JY, Rietjens M, Botteri E, et al. Evaluation of fat grafting safety in patients with intraepithelial neoplasia: A matched-cohort study. *Ann Oncol*. 2013;24:1479–1484.
19. Chaput B, Grolleau JL, Bertheuil N, Eburdery H, Chavoïn JP, Garrido I. Another suspected case of breast cancer recurrence after lipofilling? Remain cautious *J Plast Reconstr Aesthet Surg*. 2014;67:1156–1157.
20. Smit JM, Tielemans HJ, de Vries B, Tuinder SM. Recurrence of invasive ductal breast carcinoma 10 months after autologous fat grafting. *J Plast Reconstr Aesthet Surg*. 2014;67:e127–e128.
21. Orbay H, Devi K, Williams PA, Dehghani T, Silva EA, Sahar DE. Comparison of endothelial differentiation capacities of human and rat adipose-derived stem cells. *Plast Reconstr Surg*. 2016;138:1231–1241.
22. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 2012;9:671–675.
23. Donnenberg VS, Zimmerlin L, Rubin JP, Donnenberg AD. Regenerative therapy after cancer: What are the risks? *Tissue Eng Part B Rev*. 2010;16:567–575.
24. Jain RK, Au P, Tam J, Duda DG, Fukumura D. Engineering vascularized tissue. *Nat Biotechnol*. 2005;23:821–823.
25. Rajan A, Eubanks E, Edwards S, et al. Optimized cell survival and seeding efficiency for craniofacial tissue engineering using clinical stem cell therapy. *Stem Cells Transl Med*. 2014;3:1495–1503.
26. Schäffler A, Schölmerich J, Buechler C. Mechanisms of disease: Adipokines and breast cancer. Endocrine and paracrine mechanisms that connect adiposity and breast cancer. *Nat Clin Pract Endocrinol Metab*. 2007;3:345–354.
27. Freese KE, Kokai L, Edwards RP, et al. Adipose-derived stem cells and their role in human cancer development, growth, progression, and metastasis: A systematic review. *Cancer Res*. 2015;75:1161–1168.
28. Key TJ, Appleby PN, Reeves GK, et al.; Endogenous Hormones Breast Cancer Collaborative Group. Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women. *J Natl Cancer Inst*. 2003;95:1218–1226.
29. Donnenberg VS, Donnenberg AD, Zimmerlin L, et al. Localization of CD44 and CD90 positive cells to the invasive front of breast tumors. *Cytometry B Clin Cytom*. 2010;78:287–301.
30. Donnenberg VS, Luketich JD, Landreneau RJ, DeLoia JA, Basse P, Donnenberg AD. Tumorigenic epithelial stem cells and their normal counterparts. *Ernst Schering Found Symp Proc*. 2006;5:245–263.
31. Delay E, Garson S, Tousson G, Sinna R. Fat injection to the breast: Technique, results, and indications based on 880 procedures over 10 years. *Aesthet Surg J*. 2009;29:360–376.
32. Gale KL, Rakha EA, Ball G, Tan VK, McCulley SJ, Macmillan RD. A case-controlled study of the oncologic safety of fat grafting. *Plast Reconstr Surg*. 2015;135:1263–1275.
33. Pérez-Cano R, Vranckx JJ, Lasso JM, et al. Prospective trial of adipose-derived regenerative cell (ADRC)-enriched fat grafting for partial mastectomy defects: The RESTORE-2 trial. *Eur J Surg Oncol*. 2012;38:382–389.
34. Charvet HJ, Orbay H, Wong MS, Sahar DE. The oncologic safety of breast fat grafting and contradictions between basic science and clinical studies: A systematic review of the recent literature. *Ann Plast Surg*. 2015;75:471–479.
35. Veronesi U, Cascinelli N, Mariani L, et al. Twenty-year follow-up of a randomized study comparing breast-conserving surgery with radical mastectomy for early breast cancer. *N Engl J Med*. 2002;347:1227–1232.
36. Fisher B, Anderson S, Bryant J, et al. Twenty-year follow-up of a randomized trial comparing total mastectomy, lumpectomy, and lumpectomy plus irradiation for the treatment of invasive breast cancer. *N Engl J Med*. 2002;347:1233–1241.
37. Yoshimura K, Sato K, Aoi N, Kurita M, Hirohi T, Harii K. Cell-assisted lipotransfer for cosmetic breast augmentation: Supportive use of adipose-derived stem/stromal cells. *Aesthetic Plast Surg*. 2008;32:48–55; discussion 56–57.
38. Matsumoto D, Sato K, Gonda K, et al. Cell-assisted lipotransfer: Supportive use of human adipose-derived cells for soft tissue augmentation with lipoinjection. *Tissue Eng*. 2006;12:3375–3382.