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Adipose-Derived Stromal Vascular Fraction Differentially Expands Breast Progenitors in Tissue Adjacent to Tumors Compared to Healthy Breast Tissue

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Background: Autologous fat grafts supplemented with adipose-derived stromal vascular fraction are used in reconstructive and cosmetic breast procedures. Stromal vascular fraction contains adipose-derived stem cells that are thought to encourage wound healing, tissue regeneration, and graft retention. Although use of stromal vascular fraction has provided exciting perspectives for aesthetic procedures, no studies have yet been conducted to determine whether its cells contribute to breast tissue regeneration. The authors examined the effect of these cells on the expansion of human breast epithelial progenitors.

Methods: From patients undergoing reconstructive breast surgery following mastectomies, abdominal fat, matching tissue adjacent to breast tumors, and the contralateral non–tumor-containing breast tissue were obtained. Ex vivo co-cultures using breast epithelial cells and the stromal vascular fraction cells were used to study the expansion potential of breast progenitors. Breast reduction samples were collected as a source of healthy breast cells.

Results: The authors observed that progenitors present in healthy breast tissue or contralateral non–tumor-containing breast tissue showed significant and robust expansion in the presence of stromal vascular fraction (5.2- and 4.8-fold, respectively). Whereas the healthy progenitors expanded up to 3-fold without the stromal vascular fraction cells, the expansion of tissue adjacent to breast tumor progenitors required the presence of stromal vascular fraction cells, leading to a 7-fold expansion, which was significantly higher than the expansion of healthy progenitors with stromal vascular fraction.

Conclusions: The use of stromal vascular fraction might be more beneficial to reconstructive operations following mastectomies compared with cosmetic corrections of the healthy breast. Future studies are required to examine the potential risk factors associated with its use. (*Plast. Reconstr. Surg.* 136: 414e, 2015.)

CLINICAL QUESTION/LEVEL OF EVIDENCE: Therapeutic, V.

Approximately 22,000 breast cancer patients in Canada each year undergo mastectomy surgery to reduce their risk of tumor recurrence.[1] However, loss of breast tissue because of mastectomy could severely affect the patient's quality of life and therefore breast reconstruction procedures have

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become an important aspect of breast cancer care and treatment. Mastectomy operations lead to the distortion of the breast volume and shape, and the follow-up radiation therapy often results in breast tissue fibrosis and poor wound healing.[2–4] Autologous fat grafting has become the most common procedure for restoring breast structure, volume, and contour after mastectomy reconstructive surgery. In such procedures, typically, autologous fat tissue from the patient's abdomen is used as filler because it has shown promising results in repairing soft-tissue defects caused by tumor resection and local tissue deformities caused by surgical incision procedures.[5–12] In the case of breast augmentation, the use of silicone prostheses has been well established.[13,14] However, 20 percent of these patients are prone to developing capsular contracture and/or other long-term complications.[15,16] For this reason, autologous fat grafting is gaining popularity in aesthetic operations to provide shape and volume.[17,18]

Abdominal fat tissue consists of a heterogeneous population of cells, including a small number of adipose-derived mesenchymal stem cells. Recent observations suggest that the mesenchymal stem cells are important for tissue regeneration and homeostasis.[19,20] Besides their role in tissue development, mesenchymal stem cells have been shown to have proangiogenic and possible wound-healing properties at sites of tissues damage.[21–25] In addition, mesenchymal stem cells have been shown to secrete several growth factors such as cytokines that are important for tissue repair and maintenance.[26–30] These characteristics, combined with their extensive self-renewal capacity, make adipose-derived mesenchymal stem cells an ideal candidate to provide better wound healing in the short run and better graft maintenance in the long run.[31,32] Because of these properties, autologous fat grafting procedures have been further developed to include mesenchymal stem cell–enhanced fat grafts using the stromal vascular fraction. In the operating room, stromal vascular fraction samples are obtained from the infranatant of centrifuged lipoaspirate. Combining stromal vascular fraction and autologous fat (i.e., cell-assisted fat grafts), before the injection of the processed fat, has been shown to increase the take of autologous fat that is grafted into various body parts.[33,34] In the laboratory, the stromal vascular fraction samples are obtained through enzymatic digestion of either liposuctioned fat or abdominal fat tissue. Cell-assisted lipotransfer with stromal vascular fraction is commonly used to increase the take percentage of the fat cells. However, the potential effects of stromal vascular fraction cells and/or adipose-derived mesenchymal stem cells on the proliferation and differentiation of progenitors and stem cells that are present in the tissue adjacent to breast tumors have not been studied. Moreover, the effects of stromal vascular fraction cells on the tumor microenvironment and how they influence the proliferation of breast cancer cells or possibly de novo tumor formation remain elusive and highly controversial.[35,36]

Studies using breast cancer cell lines or pleural effusion samples from breast cancer patients have shown that co-culture with adipose-derived mesenchymal stem cells promotes growth and invasion of the breast cancer cells in vitro[37,38] and in vivo.[34,39,40] Based on these observations, it has been suggested that the plethora of cytokines, chemokines, and growth factors that are secreted by the adipose-derived mesenchymal stem cells could facilitate tumor initiation, progression, and metastasis.[41,42] Considered together, these observations suggest that the stromal vascular fraction cells along with adipose-derived mesenchymal stem cells might play an important role in breast tissue regeneration following mastectomy and cosmetic procedures; however, they may also provide an environment that supports tumor development and progression. To assess any potential risks associated with the use of stromal vascular fraction in reconstructive or aesthetic procedures, we must first determine whether stromal vascular fraction cells play a role in regenerating breast tissue in healthy individuals and in breast cancer patients. In this study, we have examined the effects of stromal vascular fraction on breast epithelial progenitor cell proliferation present in tissue adjacent to breast tumors, the matching contralateral non–tumor-containing breast tissue, and reduction mammaplasty samples using in vitro three-dimensional assays.

PATIENTS AND METHODS

Human Breast Tissue and Stromal Vascular Fraction Preparation

All tissue samples were collected based on informed, written patient consent and in compliance with research ethics board approval (REBHS14919 and REBHS210:272). From four patients undergoing reconstructive operations following mastectomies, subcutaneous abdominal fat tissues, tissues adjacent (>3 cm away) to breast tumors, and contralateral non–tumor-containing breast tissue were obtained. All primary tumors were invasive ductal carcinoma and stained positive for estrogen and progesterone receptor expression with lymph node involvement. The tissue adjacent to breast tumors and contralateral non–tumor-containing breast tissue samples were declared

disease-free by a breast pathologist. Tissue samples were also collected from discarded reduction mammaplasty tissue (four patients). All tissue samples were transported from the operating room to the laboratory in transport media (Dulbecco's Modified Eagle Medium–F12 supplemented with 5% bovine serum, insulin (5 μg/ml; Sigma-Aldrich, St. Louis, Mo.), and antibiotics (Invitrogen, Carlsbad, Calif.). The stromal vascular fraction was isolated from the fat tissues as follows: the fat samples were minced and digested for 4 hours at 37.5°C with shaking in Ham's F12–Dulbecco's Modified Eagle Medium (1:1 volume/volume F12 to Dulbecco's Modified Eagle Medium) supplemented with 2% bovine serum albumin, 300 units/ml collagenase, 100 units/ml hyaluronidase, 10 ng/ml epidermal growth factor, 1 mg/ml insulin, and 0.5 mg/ml hydrocortisone (Sigma-Aldrich). Subsequently, the released cells were pelleted (at 1200 rpm for 5 minutes) and washed with Hank's Balanced Salt Solution supplemented with 2% fetal bovine serum, and the resulting pellets were treated with red blood cell lysis buffer (Sigma-Aldrich). The cell suspension was pelleted and resuspended in fetal bovine serum and 7% dimethylsulfoxide solution and stored cryogenically. The breast tissue samples were digested and processed as described before.[43]

Colony-Forming Cell Assays

The colony-forming unit–fibroblast assays were set up using single-cell suspensions from freshly defrosted stromal vascular fraction samples as described[44] and plated onto tissue culture plates (5, 2, 1, or 0.5×10^3 cells/plate) using complete MesenCult medium (Stem Cell Technologies, Inc., Vancouver, British Columbia, Canada). The cultures were maintained in an incubator with 5% carbon dioxide for 14 days. Subsequently, the colonies were fixed in methanol/acetone and stained with crystal violet. The colony numbers were obtained using an inverted microscope. To set up the breast epithelial cell colony-forming cell assays, the breast samples were made into single-cell suspensions as described,[45] and 5000 cells were combined with 70,000 irradiated mouse embryonic fibroblasts in SF-7 growth media[46] supplemented with 5% fetal bovine serum. After 10 days, the colonies were fixed and stained with crystal violet and the colony numbers were ascertained as described. Colonies types were distinguished using immunofluorescent staining for differentiated luminal and myoepithelial cells.

Flow Cytometry

The stromal vascular fraction samples were analyzed for the presence of adipose-derived mesenchymal stem cells using flow cytometry (Guava 8HT; Millipore, Billerica, Mass.). To obtain enough cells for flow cytometry, cultured stromal vascular fractions were used at passage 0 after cells reached 75 percent confluence. Single-cell suspensions from passage 0 cells were stained with mouse anti-human CD14–fluorescein isothiocyanate (1 μg/ml), CD19–fluorescein isothiocyanate (1 μg/ml), CD90-fluorescein isothiocyanate (1 μg/ml), CD105–fluorescein isothiocyanate (1 μg/ml), CD73-phycoerythrin (1 μg/ml), CD45-phycoerythrin (1 μg/ml), CD34-phycoerythrin (1 μg/ml), and CD13-phycoerythrin (1 μg/ml) using standard protocols.[45] Mouse fluorescein isothiocyanate– or phycoerythrin-conjugated mouse immunoglobulin G1 (1 μg/ml) was used as an isotype control. Fluorescein isothiocyanate–conjugated antibodies were purchased from Serotec (Raleigh, N.C.) and phycoerythrin-conjugated antibodies were purchased from BD Biosciences (San Jose, Calif.).

Adipose-Derived Mesenchymal Stem Cell Lineage Differentiation

To assess the multilineage differentiation of adipose-derived mesenchymal stem cells in the stromal vascular fraction samples, each sample was turned into single cells and 1×10^5 cells/well were cultured in six-well plates and allowed to reach greater than 75 percent confluence. Adipose-derived mesenchymal stem cells were differentiated into adipose, cartilage, or bone using the Poietics human mesenchymal stem cells kit from Lonza (Walkersville, Md.) according to the manufacturer's protocol. Subsequently, the growth media was replaced with the adipogenic or the osteogenic media or nonsupplemented growth media as controls. The growth medium was replaced every 3 days, and after 21 days, the cells were fixed with 10% formalin and stained to detect differentiated cells. Oil Red O, Alizarin Red, and Alcian Blue 8GX stains were used to identify adipocytes, osteoblasts, and chondrocytes, respectively, according to the manufacturer's protocol (Sigma-Aldrich).

Matrigel Cultures

Single-cell suspensions obtained from the breast tissue samples were cultured in Matrigel (Becton, Dickinson & Co., Franklin Lakes, N.J.) either alone or in combination with the stromal vascular fraction cells. Then, 2×10^5 breast epithelial cells were place in Matrigel cultures using SF-7 media supplemented with bovine pituitary extract (100 μg/ml) and placed in an incubator at 37°C for 14 days. In the case of co-cultures, 1×10^5

stromal vascular fraction cells were combined with 1×10^5 breast epithelial cells and placed in Matrigel cultures as described. After 14 days, the Matrigel cultures were made into single-cell suspensions using Dispase (5 mg/ml; Stem Cell Technologies) and 0.25% trypsin/ethylenediaminetetraacetic acid (Stem Cell Technologies). The single-cell suspensions (10 percent and 30 percent of the cell suspensions) were used in colony-forming cell assays as described, and the total colony numbers were obtained by back-calculating to 100 percent of the cell suspension obtained from each gel.

Statistical Analysis

To calculate the expansion of progenitors in Matrigel cultures, the input number of progenitors was used as the denominator for each arm of the experiment and the analysis of variance was applied to ascertain statistical validity ($p \leq 0.05$). The pair-wise comparisons were performed using a two-tailed *t* test.

RESULTS

Characterization and Quantification of Adipose-Derived Mesenchymal Stem Cells in Stromal Vascular Fraction

The stromal vascular fraction samples have previously been shown to contain undifferentiated adipose-derived mesenchymal stem cells. To characterize and quantify the number of adipose-derived mesenchymal stem cells in the stromal vascular fraction samples,[47] each sample was cultured in maintenance growth medium (MesenCult). Initially, at passage 0, the cultures consisted of a phenotypically heterogeneous population of cells. However, over subsequent passages, the cultures adopted a homogeneous fibroblast-like morphology (Fig. 1). To examine the frequency of mesenchymal stem cells in the stromal vascular fraction samples, increasing numbers of cells (500, 1000, 2000, and 5000) from each sample were placed in the colony-forming unit–fibroblast assays (Fig. 2, *above*, *left*). The limiting dilution assay is performed to avoid overcrowding of the culture plates and to improve colony count accuracy. As shown in Figure 2, although there is a remarkable reproducibility within the biological replicates (Fig. 2, *above*, *right*), culturing more than 1000 cells led to decreased mesenchymal stem cell frequency (Fig. 2, *below*, *left*). This observation suggests that crowding the colony-forming unit–fibroblast plates could impact mesenchymal stem cell frequency calculations, and establishing a standard curve is necessary to accurately determine the mesenchymal stem cell frequency in the stromal vascular fraction samples. Based on these data, we estimate that approximately 2.62 ± 0.27 percent of the cells in the stromal vascular fraction samples contain adipose-derived mesenchymal stem cells (Fig. 2, *below*, *left*). Moreover, the colony-forming efficiency of adipose-derived mesenchymal stem cells decreases significantly at passages 3 and 4 compared with the freshly isolated (passage 0) and passage 1 or 2 stromal vascular fraction cells (Fig. 2, *below*, *right*).

Next, we examined the expression of mesenchymal stem cell markers[47] in the stromal vascular fraction samples using flow cytometry and found that that more than 90 percent of cultured stromal

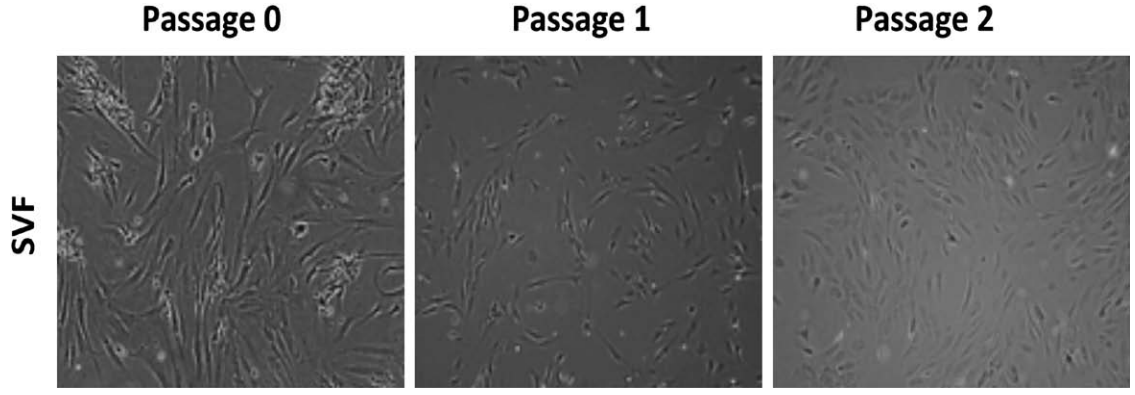

Fig. 1. Derivation and maintenance of human adipose-derived mesenchymal stem cells from the stromal vascular fraction samples. The stromal vascular fractions (*SVF*) were made into single-cell suspensions and placed in MesenCult medium to maintain the adipose-derived mesenchymal stem cells in an undifferentiated state. At passage 0, the culture contained a heterogeneous population of cells with mixed morphology. However, at passages 1 and 2, the cultures became more homogeneous and exhibited a fibroblast-like morphology. Representative images of three different stromal vascular fraction samples from different passages are shown (original magnification, × 4).

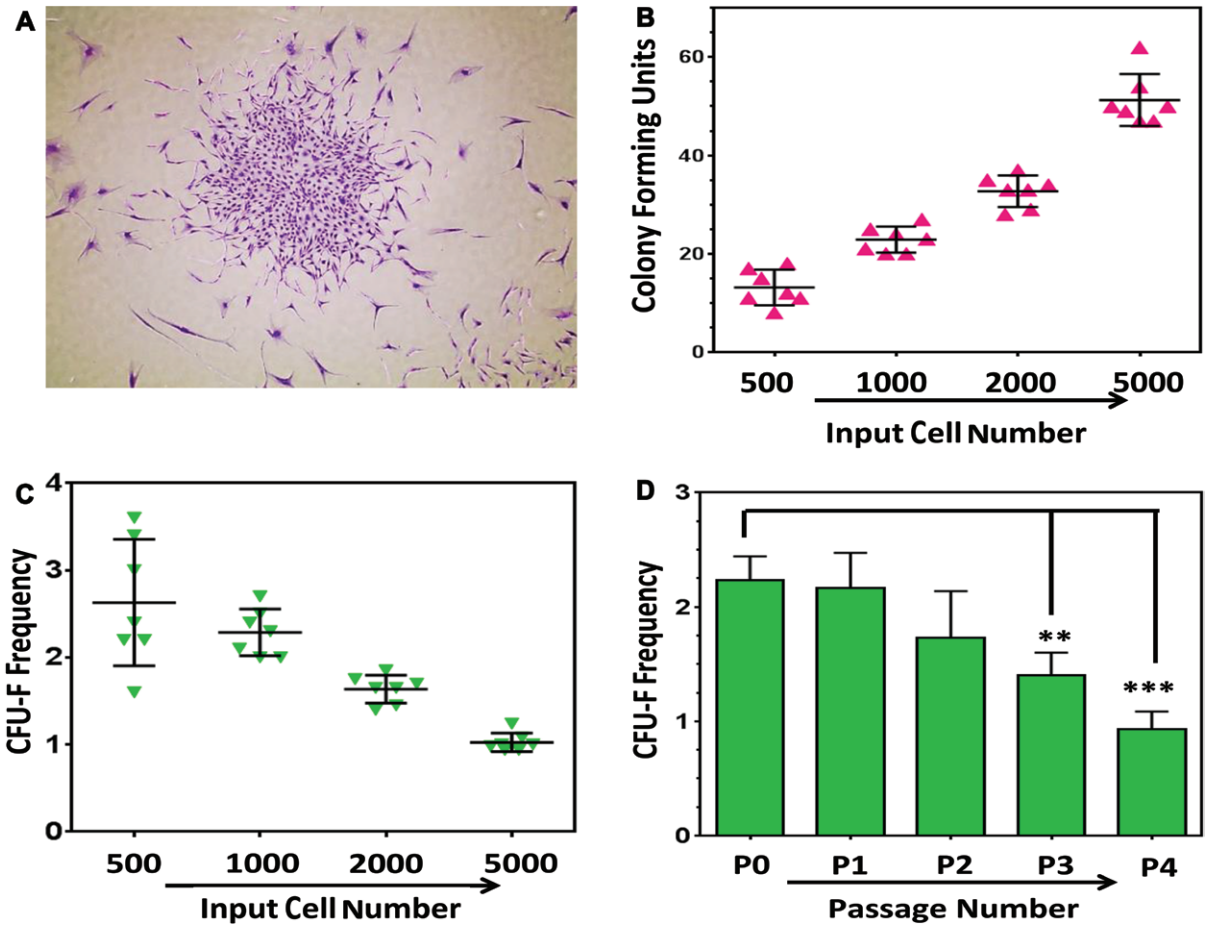

Fig. 2. The stromal vascular fraction contains adipose-derived mesenchymal stem cells. The stromal vascular fractions were made into single-cell suspensions and placed in colony-forming unit–fibroblast assays using the indicated starting cell numbers. After 14 days, colonies were fixed and stained with crystal violet. (*Above*, *left*) A representative photograph of a fibroblast colony derived from adipose-derived mesenchymal stem cells is shown (original magnification, × 4). (*Above*, *right*) The colony number is plotted against starting cell dose from different stromal vascular fraction samples ($n = 7$). As can be seen, culturing more cells will yield more colonies. (*Below*, *left*) The frequency of adipose-derived mesenchymal stem cells was calculated based on the number of colonies formed from each starting cell dose and plotted on a bar graph. As shown, at starting cell numbers over 1000 cells, the adipose-derived mesenchymal stem cell frequency is underestimated because of overcrowding of the plates, leading to less accurate colony counts. On average, only 2.62 ± 0.27 percent of each stromal vascular fraction sample contains adipose-derived mesenchymal stem cells. (*Below*, *right*) Colony-forming efficiency of adipose-derived mesenchymal stem cells in the stromal vascular fraction samples decreases at passage 3 (**$p < 0.005$) and passage 4 (***$p < 0.0005$) compared with the freshly isolated (***passage 0) samples. *CFC-F*, colony-forming cell–fibroblast.

vascular fraction cells at passage 0 express CD13, CD73, CD90, and CD105 (Fig. 3). In addition, we found that the stromal vascular fraction cells did not express CD14, CD19, CD34, or CD45 (data not shown). The expression profile of these cell surface markers is consistent with the previously established phenotypic characterization of adipose-derived mesenchymal stem cells.[20,47] These markers, however, do not sufficiently describe the adipose-derived mesenchymal stem cells in the stromal vascular fraction samples because only 2.6 percent of the stromal vascular fraction samples contain adipose-derived mesenchymal stem cells. Therefore, other markers are needed to better describe adipose-derived mesenchymal stem cells.

To assess the multilineage differentiation capacity, the stromal vascular fraction–derived cells were cultured under adipogenic, osteogenic, and chondrogenic differentiation conditions for 21 days (Fig. 4). The cells cultured in the adipogenic medium stained positively with Oil Red O, distinguishing fat droplets in cells; and cells cultured in the osteogenic medium were positively stained with the Alizarin Red S, indicative of bone matrix

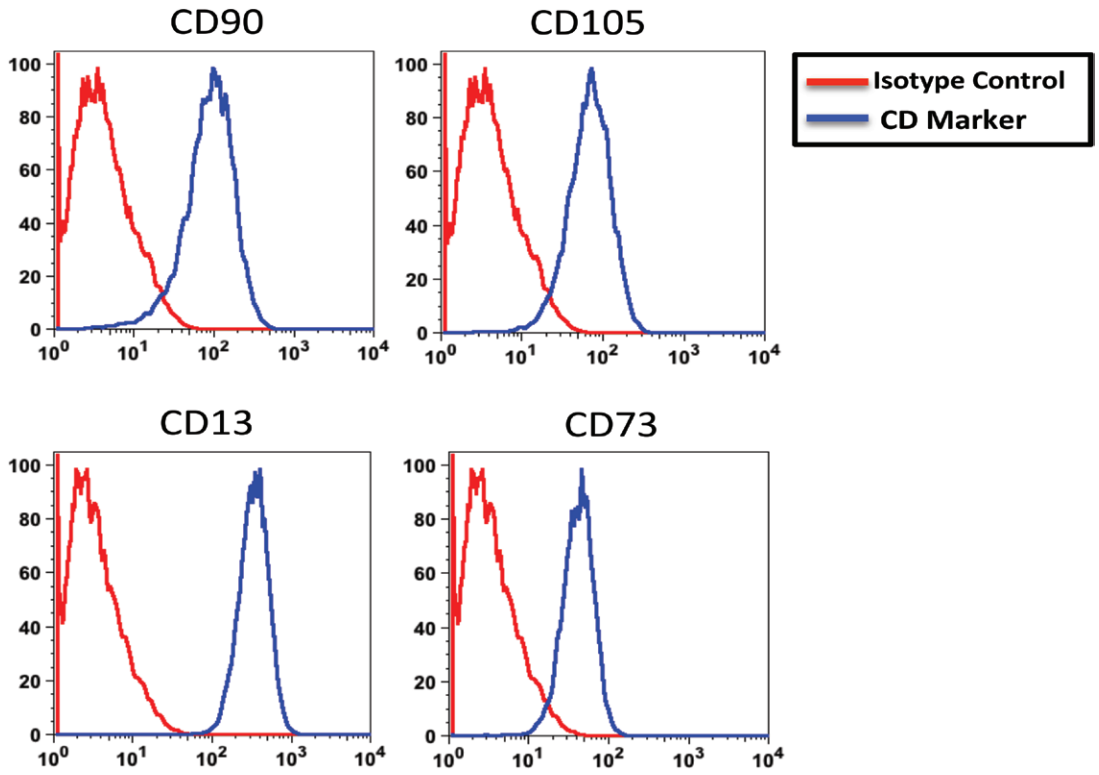

Fig. 3. Phenotypic characterization adipose-derived mesenchymal stem cells in the stromal vascular fraction. The expression of adipose-derived mesenchymal stem cell markers in the passage 0 stromal vascular fraction cells was determined by means of flow cytometry. The histogram analysis of each marker revealed that more than 90 percent of the isolated cells expressed CD13, CD90, CD73, and CD105. These data are representative of three individual experiments using three different stromal vascular fraction samples.

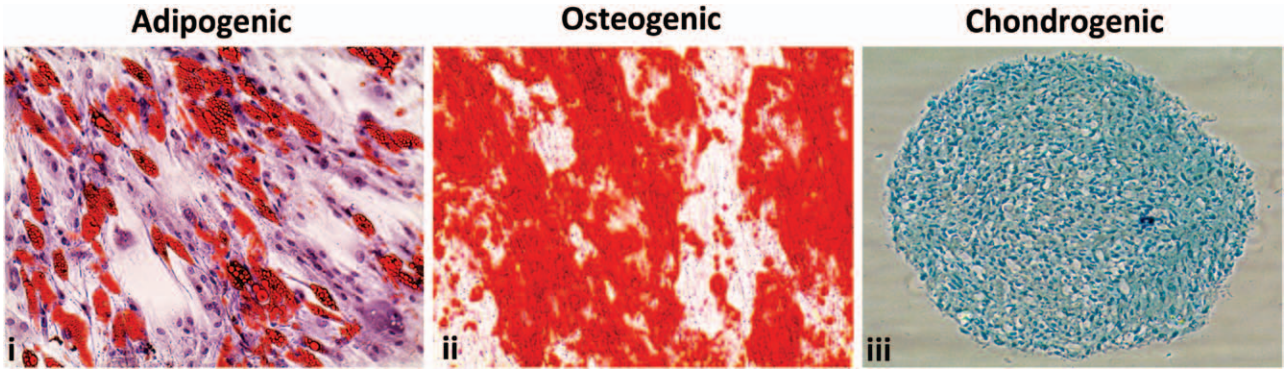

Fig. 4. Stromal vascular fraction samples contain multipotential adipose-derived mesenchymal stem cells. Multipotency of the adipose-derived mesenchymal stem cells present in the stromal vascular fractions was examined. Stromal vascular fraction cells were placed in adipogenic, osteogenic, and chondrogenic differentiation culture conditions. Cells were fixed and stained with Oil Red O to identify presence of adipocytes (*left*) or Alizarin Red S to distinguish differentiation to osteoblasts and bone matrix mineralization and calcium deposition (*center*). Cells grown in chondrogenic medium (*right*) show positive staining for Alcian Blue 8GX, indicative of chondrogenic differentiation.

mineralization and calcium deposition. Cells grown in chondrogenic medium were positively stained with Alcian Blue 8GX, indicative of acidic polysaccharides such as glycosaminoglycan found in cartilage (Fig. 4). Cells that were grown in the control maintenance medium remained undifferentiated and showed no staining for adipogenesis or osteogenesis and remained fibroblast-like (data not shown). Based on these observations, we conclude that our stromal vascular fraction samples

contain multipotential adipose-derived mesenchymal stem cells.

Stromal Vascular Fraction Cells Modestly Enhance the Expansion of Healthy Breast Epithelial Progenitors

Stromal vascular fraction–enhanced fat grafts are used in cosmetic breast operations to encourage tissue regeneration. Breast tissue regeneration requires the differentiation of breast stem cells into progenitor cells, which in turn proliferate extensively (i.e., expansion) to produce the required number of mature breast cells. To investigate whether stromal vascular fraction cells could influence the expansion potential of breast progenitors, we used the three-dimensional Matrigel culture system.[48] We previously showed that placing mouse breast cells in Matrigel cultures leads to the expansion of epithelial progenitors.[48] We therefore hypothesized that placing human cells in similar Matrigel cultures would also lead to the expansion of the human breast progenitors. Therefore, we placed breast cells from reduction mammaplasty samples in Matrigel cultures for 14 days. To quantify the starting progenitor cell number (input) we used the colony-forming cell assay where the colony numbers provide a prospective measure of the progenitor numbers. After 14 days, the Matrigel cultures were dissociated and cells were subjected to colony-forming cell assays where output number of progenitors was determined (Fig. 5). (**See Table, Supplemental Digital Content 1**, which demonstrates epithelial progenitor frequency in tissue adjacent to breast tumors and healthy breast tissue. This table shows the number of input progenitors as present in the healthy breast tissue or tissue adjacent to breast tumors as determined by the colony-forming cell assay. The output progenitor numbers from each sample cultured with or without stromal vascular fractions were obtained through the colony-forming cell assays after 14 days of Matrigel cultures. The numbers are representative of three tissues adjacent to breast tumor samples along with stromal vascular fraction samples from the same patients. Four reduction mammaplasty samples were used as the source of healthy breast tissue. The numbers represent total progenitor numbers in each individual sample based on frequency of progenitors, and total cell numbers are averages of three or four samples, ***http://links.lww.com/PRS/B383***.) Compared to the input, the progenitors in the reduction samples expanded 3.4 ± 0.57-fold ($p < 0.001$) (Fig. 6), whereas in the co-cultures with stromal vascular fraction, the progenitor cells expanded by 5.2 ± 0.52-fold, leading

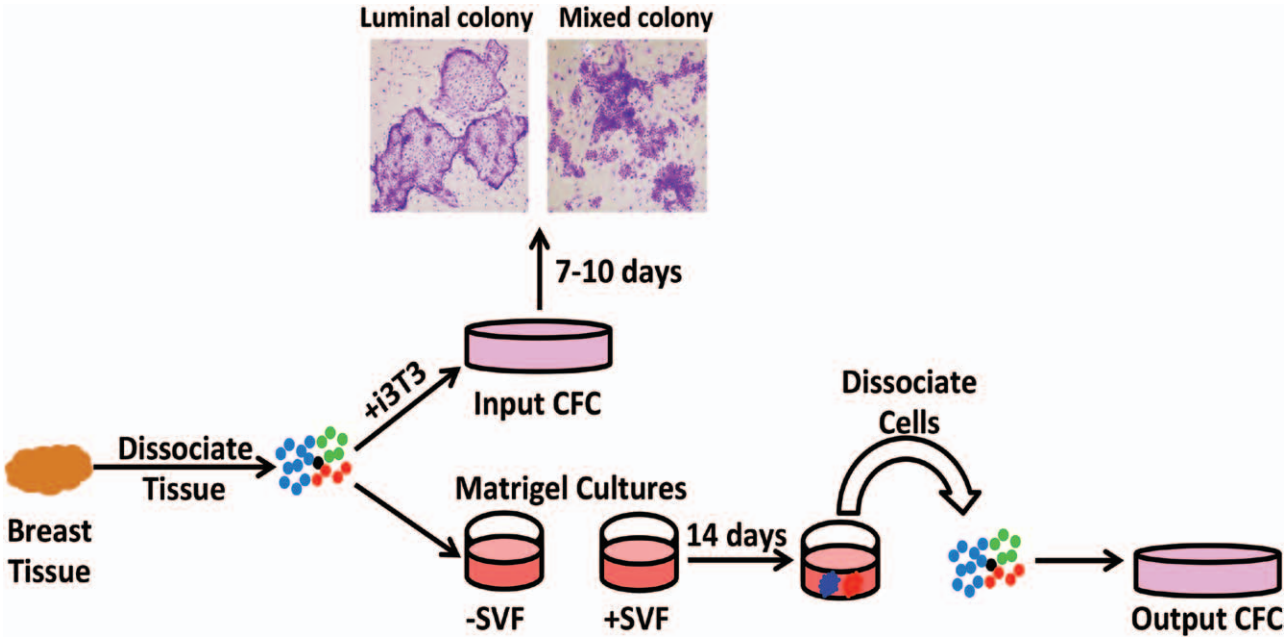

Fig. 5. Stromal vascular fraction induces extensive expansion of progenitors in tumor-adjacent breast tissue. The diagram depicts the experimental outline. Briefly, single-cells suspensions of reduction mammaplasties or tissue adjacent to breast tumors (*TABT*) or contralateral non–tumor-containing breast tissue were placed in Matrigel cultures either alone or in co-cultures with single-cell preparations of stromal vascular fractions (*SVF*). Input number of progenitors in the breast samples was ascertained using the colony-forming cell assay (*CFC*) before the Matrigel cultures were set up.

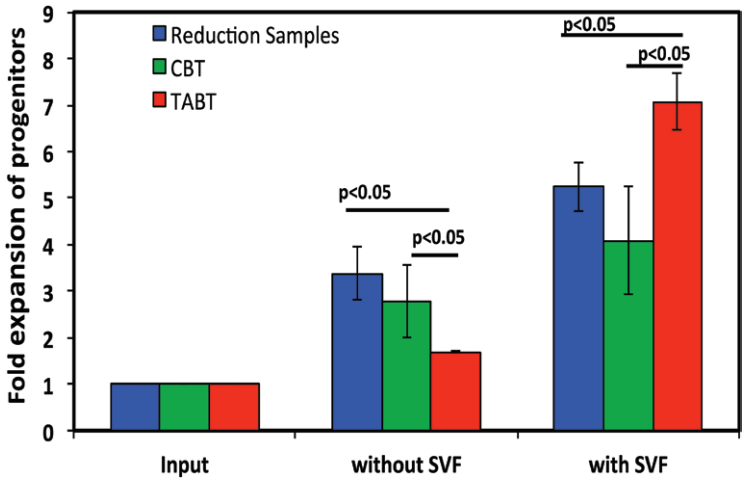

Fig. 6. After 14 days, the single-cells suspensions from the Matrigel cultures were placed in the colony-forming cell assays to quantify the output number of progenitors. Whereas the reduction mammaplasty samples and the contralateral non–tumor-containing breast tissue cells (*CBT*) expanded by 3.4-fold and 2.4-fold, respectively, the tissue samples adjacent to breast tumor progenitors (*TABT*) did not expand on their own (1.7-fold). When co-cultured with the stromal vascular fraction (*SVF*) samples, the progenitors in the breast reduction samples and the contralateral non–tumor-containing breast tissue samples expanded 5.2- and 4.1-fold. However, the tissue adjacent to breast tumor progenitors expanded 7.1-fold when placed in co-cultures with stromal vascular fraction.

to an additional 1.53-fold ($p < 0.005$) progenitor expansion (Fig. 6). Colony characterization (Fig. 7) revealed that luminal progenitors (Fig. 7, *left*, and Fig. 8, *left*) are the dominant progenitor subtype (approximately 70 percent) found in healthy breast cells. We found that placing healthy breast cells in Matrigel cultures alone or along with stromal vascular fraction did not significantly alter the distribution of the progenitor subtypes (Fig. 8, *left*). Overall, our results demonstrate that Matrigel cultures can be used to expand healthy breast progenitors and that stromal vascular fraction cells have a small effect on the expansion potential of these progenitors.

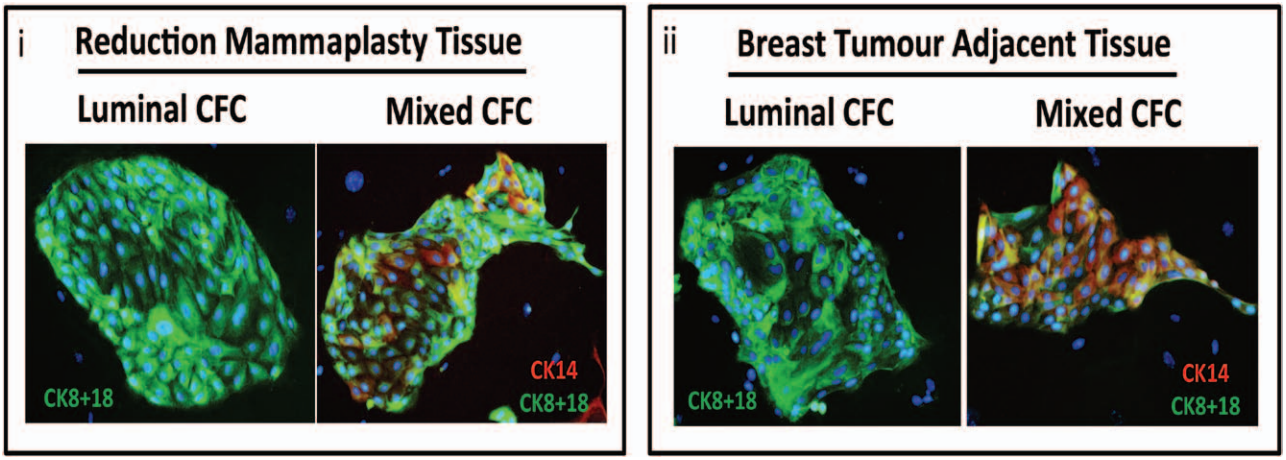

Fig. 7. Cells from both the reduction mammaplasty samples (*left*) and tissue adjacent to breast tumors (*right*) can form colonies of both luminal and mixed (containing both luminal and myoepithelial cells) lineages in the colony-forming cell (*CFC*) assays. Luminal colonies stained positive for cytokeratin 8+18 (*CK8+18*), which marks the luminal cells; whereas mixed colonies stained positively for both cytokeratin 8+18 and cytokeratin 14 (*CK14*), which mark luminal and myoepithelial cells, respectively.

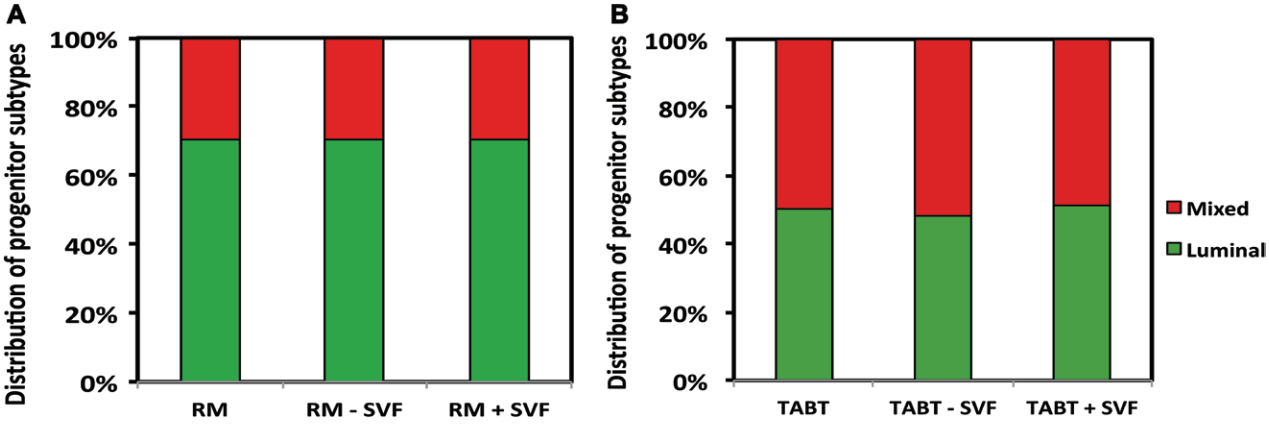

Fig. 8. (*Left*) The distribution of different progenitor subtypes in Matrigel cultures with and without stromal vascular fraction (*SVF*) was determined by the colony types. In the case of the reduction mammaplasty (*RM*) samples, the majority of the colonies were of luminal subtype (approximately 70 percent), which was not altered when reduction mammaplasty cells were placed in stromal vascular fraction co-cultures. (*Right*) When the tissue adjacent to breast tumor (*TABT*) samples where place in Matrigel assays, they showed an equal distribution of the luminal and the mixed colonies, which was also observed in the co-cultures with the stromal vascular fraction cells. The results are representative of three independent samples.

Stromal Vascular Fraction Cells Significantly Enhanced the Expansion of Progenitors in Tumor-Adjacent Breast Cells

The use of stromal vascular fraction along with the fat tissue in reconstructive procedures following mastectomy operations is gaining popularity because it is thought to enhance healing and graft maintenance.[8–12] However, no information exists about the potential effects of stromal vascular fraction cells on regeneration of the tissue adjacent to breast tumors. We therefore used our Matrigel cultures to determine whether co-cultures of tissue adjacent to breast tumors cells and stromal vascular fraction cells would lead to enhanced expansion potential of progenitors (Fig. 5). As controls, we used matching contralateral non–tumor-containing breast tissue samples obtained from the same patients. Similar to the reduction mammaplasty samples, the contralateral non–tumor-containing breast tissue progenitors on their own showed a 2.4 ± 0.8-fold expansion and with the stromal vascular fraction cells they showed a 4.1 ± 1.2-fold expansion in the Matrigel assays (Fig. 6).

In contrast to the contralateral non–tumor-containing breast tissue samples, culturing the tissue adjacent to breast tumor samples in Matrigel did not result in a significant increase (1.7 ± 0.03-fold) in the number of progenitors (Fig. 6). However, the co-cultures of tissue adjacent to breast tumor cells and stromal vascular fraction led to a significant ($p < 0.05$) increase in progenitors (7.1 ± 0.6-fold) (Fig. 6). Unlike the reduction mammaplasty samples, where luminal progenitors were more prevalent than the bipotential progenitors, the tissue adjacent to breast tumor samples consisted of an equal ratio of luminal progenitors to bipotential progenitors, and this ratio was maintained in Matrigel cultures with or without stromal vascular fraction (Fig. 7, *right*, and Fig. 8, *right*). These data suggest that progenitors from tissue adjacent to breast tumors are more reliant on signals from their environment to proliferate and differentiate compared with healthy progenitors. Moreover, our data suggest that stromal vascular fraction has a greater effect on expanding progenitors from tissue adjacent to breast tumors compared with healthy progenitors.

DISCUSSION

The supplementation of fat grafts with stromal vascular fraction in breast reconstructive surgery has been gaining popularity because of its proposed role in increasing the viability of the graft and its contribution to wound healing.[5–12] However, the use of stromal vascular fraction in such operations remains controversial, as recent studies have suggested that adipose-derived mesenchymal stem cells can induce breast cancer cell proliferation in vitro and in vivo.[34,37–40] In a recent study, Duss et al.[49] reported that co-cultures of nontransformed primary human breast epithelial cells and mesenchymal precursors maintain their proliferation and differentiation potentials, and constrain their overall growth. To the best of our knowledge, no studies have been conducted to examine the effect of transplanted stromal vascular fraction cells on

the proliferation of breast epithelial cells that reside adjacent to breast tumors. Understanding the influence of stromal vascular fraction cells on tissue adjacent to breast tumor progenitor functions is a necessary first step toward studying the potential role of stromal vascular fraction in breast tissue regeneration and the potential biosafety of using stromal vascular fraction samples in breast reconstruction and other cosmetic operations. To this end, we used a three-dimensional culture system to investigate the influence of stromal vascular fraction cells on breast epithelial progenitors present in the healthy breast tissue (reduction mammaplasties) or tissue adjacent to breast tumors or matching contralateral non–tumor-containing breast tissue. Interestingly, we found that unlike healthy breast or contralateral non–tumor-containing breast tissue cells, stromal vascular fraction cells were required for the expansion of tissue adjacent to breast tumor progenitors. Moreover, tissue adjacent to breast tumor progenitors showed a much larger expansion potential compared with the healthy progenitors when placed in co-cultures with the stromal vascular fraction cells. In the clinic, stromal vascular fraction is freshly isolated in the operating room and is combined with aspirated fat tissue. We therefore chose to use unseparated stromal vascular fraction cells in this study to maintain the clinical relevance of our findings. The clinical relevance of our findings is also enhanced by the fact that we used stromal vascular fraction, tissue adjacent to breast tumors, and contralateral non–tumor-containing breast tissue from matching patients. Because of our observation that only 2.6 percent of the stromal vascular fraction cells are adipose-derived mesenchymal stem cells, it is difficult to conclude that the stromal vascular fraction–induced progenitor cell expansion is attributable to the action of adipose-derived mesenchymal stem cells alone. It will be interesting to examine the influence of different cell types that make up the stromal vascular fraction separately on the expansion of the breast progenitors.

CONCLUSIONS

Our study provides the first evidence that tissues adjacent to breast tumors have a need for a particular niche that is provided by the stromal vascular fraction cells and that stromal vascular fraction cells do not add significant benefit to the expansion of healthy progenitors. Our observations then suggest that supplementation of fat grafts with stromal vascular fraction might be more beneficial to reconstructive surgery following mastectomy compared with cosmetic procedures involving healthy breast tissue. In this study, we did not examine the biosafety concerns associated with the use of stromal vascular fraction. In most mastectomies, there are remnants of breast tissue left in proximity to the remaining skin flaps. Thus, even subcutaneous stromal vascular fraction or stromal vascular fraction–supplemented fat injections could stimulate growth in the residual breast cells. This observation can be extended to lumpectomy procedures as well. Although in the short term, stromal vascular fraction supplementation of fat grafts may be beneficial with respect to enhancing fat graft survival and inducing regeneration of breast tissue, the long-term effects of expanding breast progenitors remains uncertain and needs further investigation. It is noteworthy that the remaining breast tissue near the skin flaps in mastectomies and the tissue remaining after lumpectomy procedures could contain small and undetectable tumors, which may also be stimulated to grow in the presence of stromal vascular fraction–supplemented fat. Furthermore, in this study, we did not examine the influence of lipoaspirate (unprocessed fat) on the expansion of the breast progenitors. The concern is that, because lipoaspirate contains adipose-derived mesenchymal stem cells, its use may have an effect on the expansion of tissue adjacent to breast tumors similar to that of the stromal vascular fraction samples. Also, the effects of lipoaspirates, stromal vascular fraction, and stromal vascular fraction–supplemented lipoaspirates on tumor cell growth needs to be studied in detail.

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