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White Adipose Tissue Cells Are Recruited by Experimental Tumors and Promote Cancer Progression in Mouse Models

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Abstract

The connection between obesity and accelerated cancer progression has been established, but the mediating mechanisms are not well understood. We have shown that stromal cells from white adipose tissue (WAT) cooperate with the endothelium to promote blood vessel formation through the secretion of soluble trophic factors. Here, we hypothesize that WAT directly mediates cancer progression by serving as a source of cells that migrate to tumors and promote neovascularization. To test this hypothesis, we have evaluated the recruitment of WAT-derived cells by tumors and the effect of their engraftment on tumor growth by integrating a transgenic mouse strain engineered for expansion of traceable cells with established allograft and xenograft cancer models. Our studies show that entry of adipose stromal and endothelial cells into systemic circulation leads to their homing to and engraftment into tumor stroma and vasculature, respectively. We show that recruitment of adipose stromal cells by tumors is sufficient to promote tumor growth. Finally, we show that migration of stromal and vascular progenitor cells from WAT grafts to tumors is also associated with acceleration of cancer progression. These results provide a biological insight for the clinical association between obesity and cancer, thus outlining potential avenues for preventive and therapeutic strategies.

Introduction

Identification of factors predisposing patients to cancer progression is critical for devising preventive and therapeutic approaches. An established predisposing factor is obesity, an increasingly widespread medical problem that is associated with several life-threatening diseases (1). Epidemiologic studies have shown that obesity, defined as a body mass index (BMI) of 30 kg/m^2 , correlates with progression of many types of cancer (2, 3). Given the prevalence of overweight cancer patients, it is critical to identify the mechanisms that mediate tumor progression in obesity. Both obesity and cancer are influenced by common lifestyle elements, such as diet and physical activity (1). Although there is evidence that dietary modifications affect cancer progression, it is unclear whether this effect is direct or is secondary to the associated BMI reduction. Notably, studies in certain cancers suggest that clinical progression is accelerated in obese patients irrespective of diet (4). Because obesity

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

is manifested by overgrowth of white adipose tissue (WAT), it has been proposed that WAT itself may have a direct effect on cancer progression (5,6).

Tumor growth relies on blood vessel formation. Although tumor vasculature has been long thought to originate exclusively from preexisting adjacent vessels (“angiogenesis”), recruitment of mature and progenitor cells from remote organs may also play a role (7, 8). Indeed, circulating endothelial progenitors, circulating endothelial cells, and several distinct monocytic populations have been implicated in pathologic neovascularization (9). The relevance of this type of tumor blood vessel formation (“vasculogenesis”) has been shown in animal models: circulating bone marrow-derived progenitors engraft in the tumor vasculature and promote cancer progression (10, 11). Although differences between human disease and experimental models with respect to clinical relevance of vasculogenesis still remain to be reconciled (12), endothelial cells are systemically mobilized in human cancer (8, 9) and do engraft into tumor vessels (13), suggesting a role in disease progression. In addition to endothelial/myeloid progenitors, tumors rely on the recruitment of stromal/pericyte progenitor cells (14, 15). Accumulating evidence indicates that mesenchymal stromal cells (MSC) represent a source of progenitors with pathologic potential (16). MSC, originally characterized in bone marrow, and lately discovered in most adult organs, can differentiate into cells of mesenchymal origin, such as osteoblasts, chondrocytes, and adipocytes (17,18). MSCs are normally present in the peripheral circulation at low frequency; however, pathologic signals such as hypoxia and inflammation can lead to MSC mobilization and migration (19, 20). Recently, the capacity of MSC to “sense” cancer as a site of injury or inflammation and home to tumors has been revealed (21). Based on this, MSCs have been proposed as progenitors of the tumor stromal cells, commonly termed cancer-associated fibroblasts (CAF; refs. 14, 22).

Although bone marrow has become accepted as a source of progenitor cells, there is also evidence against the recruitment of bone marrow endothelial progenitors by tumors (12, 13). Other organs, including WAT, have not as yet been well investigated for their contribution to the circulating progenitor populations. Although data on a WAT-derived cellular contribution to cancer are lacking, experiments in rodents have shown that nonbone marrow-derived progenitors contribute to postnatal neovascularization (23). Moreover, resident endothelial precursors derived from WAT have been shown to mediate revascularization of injured mouse skeletal muscle tissue (24). WAT is composed of several cell types, including adipocytes, preadipocytes, endothelial cells, pericytes (mural cells), and other stromal cells, as well as infiltrating monocytes/macrophages (25). Clinically, expansion of WAT leading to obesity results from adipocyte hypertrophy, as well as hyperplasia driven by proliferation of progenitor cells (26, 27). Mesenchymal and endothelial progenitor cells reside in the stromal/vascular fraction (SVF) of WAT (28, 29). The population of CD45[−]CD31[−] (platelet/endothelial cell adhesion molecule 1) adipose stromal cells (here referred to as ASC) comprises the majority of cells in the SVF and displays multipotency and proliferative capacity comparable with those of bone marrow MSC while having certain unique features (28, 30). Recently, our results, confirmed by other investigators, showed that ASCs display characteristics of pericytes and promote blood vessel formation (30–32) at least partially via trophic effects of ASC-secreted vascular endothelial growth factor and hepatocyte growth factor (33 34).

According to our hypothesis (35), WAT-derived cells unbalanced by chronic inflammation in obesity (36) can respond to cancer signals, become systemically mobilized and recruited by tumors, and may promote cancer vascularization. Because tumor growth is accelerated by obesity in rodents (37), mice serve as an experimental animal suitable to study the interplay between obesity and cancer. We show that ASC and adipose endothelial cell (AEC) can migrate to tumors where they engraft into their respective compartments. Next, by taking

advantage of cells derived from a mouse strain designed for unlimited culture expansion of immortalized cells expressing enhanced green fluorescent protein (GFP), we show that migrating ASC can promote tumor growth. Finally, a newly established experimental model for tracking mobilization of WAT-derived cells *in vivo* shows that adipose progenitors can be recruited by cancer from endogenous WAT. Together, these results have implications for the mechanistic understanding of obesity in the setting of cancer.

Materials and Methods

Mouse strains

Animal experimentation was approved by the Animal Welfare Committee of the University of Texas. Tg(CAG-EGFP)B5Nagy/J “GFP mice” (38) and Tg(CAG-EGFP)B5Nagy/J, B6.Cg-Tg(ACTB-mRFP1) 1F1Hadj/J “RFP mice” (39) were from Jackson (stocks 00315 and 005884). The “Immorto/GFP” strain was created by crossing GFP mice with H-2K (b)-tsA58 “Immortomice” (40). BALB/c, C57BL/6, and NU/NU-Foxn1^{nu} (nude) mice were from Charles River.

Cell isolation and culture

Cell lines LLC, KS1767, MDA-231, and DU145 were from the American Type Culture Collection. Cell line 4T1.2 was a gift from R. Anderson (Peter MacCallum Cancer Centre, Melbourne, Australia). All cell lines were cultured in DMEM containing 10% FCS (“DMEM/FCS”). Tissues of mice with diet-induced obesity (41) were used for cell isolation as described (30, 42). The SVF fraction was separated into individual cell populations by FACSaria (BD Biosciences) with the following IgG clones: PE-anti-CD34 MEC14.7, APC-anti-CD31 MEC 13.3, PE-Cy7-anti-CD29 HM B1-1, and APC-Cy7-CD45 30-F11 (BD Biosciences). AECs were cultured in EGM-2MV (Cambrex) on fibronectin; ASC and control stromal cells (mixed population from lung and a clone from bone) were cultured in DMEM/FCS. GFP⁺ cells among peripheral blood mononuclear cells (PBMC) were enumerated with LSR II (BD Biosciences).

Transplantation and cell administration

Tumors were grafted by injecting 10⁶ cells in 100 μ L PBS s.c. onto upper back GFP⁺ cells were injected in 100 μ L PBS i.v. into tail vein or s.c. For metronomic s.c. ASC administration, the injection side on lower back was alternated daily. For WAT implantation, harvested visceral WAT of GFP mice was implanted as described (43).

Tissue analysis

Tumor size was measured with a caliper, and volume was calculated as length $\times$ width² $\times$ 0.5 (44). Tissues were recovered from anesthetized mice after heart perfusion with 10 mL PBS. Immunolocalization of GFP⁺ cells in formalin-fixed paraffin-embedded tissue sections was performed on antigen retrieval, washing with 0.2% Triton X-100, blocking in Serum-Free Protein Block (DAKO), and incubation with primary antibodies (4°C, 12–16 h) and secondary antibodies (room temperature, 1 h) in PBS containing 0.05% Tween 20. The primary antibodies used were as follows: goat anti-GFP from GeneTex (1:300), rabbit anti-RFP from Abcam (1:100), rabbit anti-CD31 from Santa Cruz Biotechnology (1:50), rabbit anti-Ki67 from Chemicon (1:200), and mouse anti- α -smooth muscle actin (α SMA) from Sigma (1:1,000). Secondary donkey FITC-conjugated (1:300) and Cy3-conjugated (1:500) IgG were from Jackson ImmunoResearch. Nuclei were stained with Hoechst 33258 or TOPRO3 (Invitrogen). Images were acquired with a confocal Leica TCS SP5 microscope/LAS AF software or Olympus IX70 inverted fluorescence microscope/MagnaFire software.

Results

Mouse adipose SVF cells home to tumors

To determine whether WAT-derived cells can be recruited by tumors, we used mice that ubiquitously express GFP (38) termed GFP mice. GFP⁺ cells of the SVF recovered from WAT of GFP mice were analyzed by flow cytometry with antibodies against CD34 marking a subset of stromal and endothelial cells, CD31 marking endothelial cells, CD45 marking hematopoietic cells, and CD29 marking stromal cells (Supplementary Table S1). Multicolor flow cytometry indicated that, after culture on plastic for 3 days and subsequent removal of non-adherent hematopoietic cells, the predominant population was comprised by CD31⁻CD45⁻CD29⁺ ASC (84.4%), expression of CD34 on a subpopulation of which was down-regulated in culture, as previously reported (28). The two other major populations of attached cells were CD31⁺CD45⁻ AEC, which added up to 2.1%, and CD45⁺ macrophages/monocytes, which constituted 7.9% of the adherent SVF.

To explore short-term homing and engraftment capability of adipose cells, 10⁶ single-passage ASC-enriched GFP-marked SVF cells were i.v. injected into BALB/c mice bearing s.c. mammary EF43. fgf4 tumor cell allografts. Mice were sacrificed at 30 minutes, 1 hour, 1 day, and 3 days after injection, and sections were evaluated for the presence of GFP⁺ cells. At all time points, 50 to 100 GFP⁺ cells per cm² were detected in tumor sections (Fig. 1A). Consistent with previous reports on MSC recruitment by tumors (20), at days 1 to 3 most GFP⁺ cells localized primarily within the tumor capsule (8). Lungs, an established site of nonspecific trapping of i.v.-injected cells, contained occasional (1–5 per cm²) GFP⁺ cells at all time points (Fig. 1A). No GFP⁺ cells were detected in other control organs at any of these time points (Fig. 1A). These results indicate that systemically administered SVF cells can home to tumors and survive up to 3 days despite the unmatched genetic background of the donor cells. To confirm that the tumor homing of adipose cells is not a model-specific artifact, we have reproduced these results in Lewis lung carcinoma (LLC) in C57BL/6 mice and 4T1.2 mammary carcinoma in BALB/c mice.

Although CD45⁺ monocytes constituted only a small fraction of the injected SVF cells, we considered a possibility that they could correspond to the cells homing to tumors due to a genetic background mismatch. However, whereas cultured ASC and AEC express high level of GFP, initially GFP⁺ monocytes/macrophages isolated from WAT of GFP mice by fluorescence-activated cell sorting (FACS) were found to completely lose GFP expression in culture (Supplementary Fig. S1). Therefore, although recruitment of monocytes by tumors is likely to occur (9, 10), the model chosen here does not allow us to detect this population in short-term transplantation experiments. To confirm that the immune reaction against the host tumor is not a mechanism behind homing of SVF cells to tumors, we repeated these experiments in a syngeneic model. The SVF cells (10⁶) from a strain that ubiquitously expresses red fluorescent protein (RFP) reporter (39) were injected into mice with the matched genetic background (C57BL/6) bearing LLC tumors. As above, immunofluorescence analysis of tissues at several time points after injection showed lodgment of RFP⁺ cells in tumors predominantly close to or within the capsule (Supplementary Fig. S2). In contrast, on injection of a matching numbers of cells isolated from lungs via a protocol analogous to WAT SVF isolation (here termed “lung SVF”), only occasional RFP⁺ cells were observed in tumors. Neither WAT- nor lung-derived SVF cells were found in other control organs (Supplementary Fig. S2), confirming tumor-homing selectivity of WAT cells.

To analyze homing of adipose cells to tumors based on human cells, we established s.c.-implanted Kaposi sarcoma (KS1767), breast adenocarcinoma (MDA-231), and prostate adenocarcinoma (DU145) xenografts in immunodeficient “nude” mice. In contrast to the

tumor allograft capsule-associated localization trend, GFP⁺ cells tended to distribute throughout the tumor in all three xenograft models (Fig. 1B). Some of the GFP⁺ cells seemed to contain multiple nuclei (Fig. 1C), which could result from cell fusion (45). Analysis of tissues with anti-Ki67 antibodies showed that GFP⁺ cells were predominantly quiescent, as only rare proliferating (Ki67⁺) GFP⁺ cells were detected (Fig. 1D). Comparison of the frequencies for WAT and lung SVF cells in tissues showed that in each case, ~50 to 100 GFP⁺ cells per cm² were observed in KS1767 tumors and 1 to 5 GFP⁺ cells per cm² were observed in lungs (Supplementary Fig. S3). As in immunocompetent mice, neither WAT- nor lung-derived SVF cells were found in other control organs.

To analyze biodistribution of injected cells by an independent method, we used a quantitative *ex vivo* replating assay based on enzymatic tissue digestion. One day after injection of 10⁶ GFP⁺ WAT SVF cells, tissue cell suspensions were cultured for 6 hours, after which attached GFP⁺ cells were counted to enumerate donor cells (Supplementary Fig. S4A). GFP⁺ cells were observed among the attached KS1767 xenograft-derived cells at a frequency of 0.5% ± 0.3%. Provided 10⁸ cells per tissue were plated in this assay, our data show that ~5 × 10⁵ (50%) of the injected cells are recruited by tumor. This is consistent with only occasional GFP⁺ cells (<0.01% among attached cells) that were recovered from lungs, thus corresponding to ~1% of injected ASC cells lodged in the lungs. No GFP⁺ cells were recovered from other organs analyzed, consistent with the immunolocalization data. Combined, these results indicate that the majority of injected WAT SVF cells home to tumors in models based on mouse and human cancer cells in both immunocompetent and immunodeficient mice.

Adipose SVF cells engraft into distinct tumor compartments

We next explored long-term lodgment of ASC by injecting 10⁶ single-passage adipose SVF cells i.v. into mice bearing KS1767 xenografts. We analyzed tissues 1 and 2 weeks after injection. At both time points, >80% of the GFP⁺ cells in tumors were localized within the stroma, suggesting their ASC identity, whereas the rest of the GFP⁺ cells seemed associated with the vasculature. At day 7, ~25% of small tumor vessels were found to contain threads of GFP⁺ cells, which by day 14 have acquired the morphology of microvessels undistinguishable from the rest of the tumor capillaries (Fig. 2). To identify the precise location of adipose cells relative to the vascular wall, we costained GFP in tissues with CD31 and αSMA marking pericytes (30, 32). Confocal microscopy analysis showed that 5% to 10% of the vessel-associated GFP⁺ expressed CD31, suggesting their endothelial identity (Fig. 2A). The endogenous GFP⁻/αSMA⁺ pericytes were observed as perivascular cells wrapped around GFP⁺ cells in capillaries (Fig. 2B), consistent with GFP⁺ cells being integrated into the lumen. Only ~1% of vessel-associated tumor-localized GFP⁺ cells were αSMA⁺ (Fig. 2B), indicating that perivascular localization of donor cells is generally not related to αSMA expression. These data show that WAT-derived cells are capable of infiltrating different tumor compartments and suggest that cells of both stromal and endothelial lineage are recruited by tumors. Confocal microscopy analysis of lungs from the same mice showed that WAT-derived cells occasionally entrapped in lungs are CD31⁻ and do not become incorporated into the lumen (Supplementary Fig. S4B), suggesting that cancer is a prerequisite of vascular engraftment.

ASC and AEC independently engraft their niches in tumors

To directly test tumor engraftment capability independently for ASC and AEC, we isolated SVF cells from GFP mice and sorted them by FACS to isolate GFP⁺/CD45⁻/CD34⁺/CD31⁻ stromal and GFP⁺/CD45⁻/CD34⁺/CD31⁺ endothelial cells (Fig. 3A). These two cell populations were expanded over a single passage for 3 days (ASC) or 1 week (AEC) in DMEM/FCS and EGM2-MV media, respectively (Fig. 3B). Consistent with the established

cellular phenotypes (30), the ASC fraction was composed of fibroblast-shaped cells, whereas the AEC fraction (which took longer to attach and proliferate) was composed of smaller cells that formed networks at higher confluency. For each cell population, 2.5×10^5 cells were administered into mice carrying KS1767 xenografts. After 1, 7, and 14 days, tissues were recovered and subjected to anti-GFP/anti-CD31 immunofluorescence. At day 1, GFP⁺ AEC and ASC were found specifically in tumors, where they seemed localized inside the blood vessels. By days 7 and 14, engrafted ASC and AEC have acquired stromal and vascular localization, respectively (Supplementary Fig. S5). ASC tended to align along the endogenous GFP⁻/CD31⁺ endothelium (Fig. 3C). In contrast, many of the GFP⁺ AEC seemed incorporated into the vascular wall (Fig. 3C; Supplementary Fig. S5). Confocal Z-stack projection of median series confirmed individual cells to express both GFP and CD31, as revealed by nuclear projection in different planes (Fig. 3C).

S.c.-administered ASCs migrate to tumors through systemic circulation

Previous studies attempting to model the effect of bone marrow MSC and ASC on tumor growth have involved acute administration of single doses of cells into animals (21). We reasoned that metronomically repeated long-term treatment of experimental animals with low doses of WAT cells would better simulate the chronic pathologic recruitment of adipose cells by tumors. Because i.v. injections conventionally used for cell administration are invasive in mice, it was first necessary to establish an alternative cell delivery method for metronomic treatments. To determine whether s.c. administration route is compatible with migration of WAT cells to tumors, we injected GFP⁺ WAT SVF cells under the skin of KS1767-xenografted mice. To monitor biodistribution, we quantified the amount of circulating GFP⁺ cells in the peripheral blood by flow cytometry (Fig. 4A). Analysis of PBMC showed that s.c.-injected cells were detectable in circulation as early as 5 minutes after injection and began clearing the circulation after 6 hours (Fig. 4B). We then determined whether this route of cell administration is compatible with their homing to tumors. GFP⁺ WAT SVF cells were s.c. injected into mice bearing KS1767 xenografts proximally (within 1 cm) to the tumor and a matched cohort of mice received a s.c. injection of the cells distantly from the tumor (5 cm away) on the contralateral flank. Immunofluorescence analysis of tumors recovered 2 weeks later revealed comparable numbers of GFP⁺ cells in the stromal and perivascular compartments in all s.c.-injected and i.v.-injected mice, suggesting that ASCs home to tumors irrespective of the administration route (Fig. 4C).

ASCs promote tumor growth

To test if ASC could contribute to cancer progression, we set out to determine if their chronic recruitment by tumors is sufficient to promote experimental cancer in mice. To generate a continuous ASC supply for metronomic administration in a long-term cancer progression study, we have developed a mouse model that allowed us to establish a consistent source of readily available ASC. This new mouse strain, Immorto/GFP, was created by crossing GFP mice (38) with Immortomice that ubiquitously express a temperature-sensitive SV40 T antigen (40). Cells from Immorto/GFP mice can be indefinitely cultured at permissive temperature (33°C), whereas at 37°C temperature the SV40 T is degraded, thus rendering GFP⁺ cells physiologically normal *in vivo*. To this end, we have isolated stromal cells from WAT, lungs, and bone marrow of Immorto/GFP mice, immortalized them, and propagated for >20 passages (Fig. 3; Supplementary Fig. S1).

To test the effect of ASC recruitment on cancer progression, we used nude mice, which have low amount of endogenous WAT. Daily injections of 10^4 immortalized ASC or control cells were performed s.c. (distantly to tumor) for 6 weeks starting 1 day after KS1767 xenografting. Tumor growth was monitored for ASC-injected mice, control (noninjected) mice, and mice injected with the following controls: (a) immortalized lung stromal cells

from Immorto/GFP mice, (b) immortalized bone stromal cells from Immorto/GFP mice, (c) 3T3-L1 fibroblasts, and (d) saline. After 2 weeks of treatment, tumor growth rate accelerated in ASC-injected mice but not in mice injected with control cells (Fig. 5A and B). The positive effect of ASC on tumor growth was reproduced in DU145 prostate cancer xenograft model (Fig. 5A). Analysis of tumors recovered from representative mice at week 6 revealed ASC accumulation in tumors but not in control tissues (Fig. 5C; Supplementary Fig. S6).

Migration of cells from WAT is associated with accelerated cancer progression

As a step toward understanding WAT as a source of progenitor cells in disease, we adapted a model based on implantation of WAT into nude mice, which have little endogenous WAT (43). As a source of WAT for transplantation, we used GFP mice, therefore making WAT-derived cells traceable in the host. Histologic examination indicated that at weeks 1 to 6, implants (average size, 1–2 cm³) predominantly contained anatomically normal WAT, which was confirmed by immunofluorescence showing typical cellularity and vascularization of GFP⁺ implants (Fig. 6A).

To test whether WAT cells can migrate to tumors, 10 days after GFP⁺ WAT implantation, KS1767 or LLC cells were grafted into the same animal ~2 cm away from the WAT implant. Changes in tumor size were quantified over a 5-week period for the KS1767 model and over a 2-week period for the LLC model, in which tumors became hemorrhagic and critically large at later time points (Fig. 6B). For each model, tumors grew significantly faster in mice carrying adipose implants (Fig. 6B and C), suggesting that cancer progression is facilitated by WAT. Immunofluorescence analysis of tissues from experimental WAT-grafted animals revealed numerous GFP⁺ cells within the tumors (Fig. 6D). For most GFP⁺ cells, localization was stromal or perivascular; however, in some cases, vascular localization of GFP⁺ cells was observed (Fig. 6D). These observations suggest that both ASC and AEC populations of WAT can be recruited by tumors and may contribute to cancer progression through engagement into tumor vascularization.

Discussion

Recent studies showing the recruitment of bone marrow-derived progenitor cells in cancer have shifted the paradigm of tumor vascularization, which had been originally thought to occur exclusively through local angiogenesis (8). However, the full spectrum of circulating stromal/vascular progenitors—along with their origins, as well as roles in disease—are yet to be fully understood. According to our hypothesis (35) experimentally tested here, WAT represents a source of progenitor cells alternative or complementary to bone marrow, which may at least partially explain a mechanistic basis for the observed effect of obesity on cancer progression (2, 3). We proposed that recruitment of WAT stromal and endothelial cells, excessively abundant in obese individuals, into growing tumors promotes tumor vascularization and growth.

We have tested our hypothesis in seven different mouse models of cancer: three human tumor xenograft models in immunodeficient mice and four murine tumor allograft models in immuno-competent mice. For transplantations, we have used both: genetic background-unmatched cells (from GFP mice) or syngeneic cells (from RFP mice). We used alternative approaches to analyze migration of adipose cells *in vivo*, which unequivocally showed that WAT-derived cells home to tumors irrespective of the model, unlike lung-derived cells, biodistribution of which may be model dependent. To advance beyond the recent reports on single-dose administration of acute cell doses (46, 47), we have designed new models that more realistically simulate physiologic cell recruitment relative to clinical settings. Aided by a mouse strain that we had generated for culture expansion of traceable cells, we have administered adipose cells into the s.c. compartment, after which their involvement into

tumor growth was analyzed. In another model, based on allotransplantation of traceable whole WAT into lean mice, recruitment of adipose cells by tumors was investigated. Our results indicate that in each model, adipose cells are recruited by tumors. These observations suggest that mobilization of cells from endogenous WAT may take place, resulting in their migration to and engraftment into tumors. Our recent report identifies SPARC (secreted protein, acidic and rich in cysteine) and CD29 (also known as $\beta 1$ integrin) as molecules implicated in mobilization of ASC (42). The detailed molecular pathways that govern WAT cell migration are yet to be fully characterized.

Previous attempts to quantify the extent of endothelial progenitor contribution to tumor vasculature and cancer progression have generated conflicting data (11, 12). It has also been debated if mesenchymal cells could contribute to tumor vasculature (16, 28, 47). Although vascular mimicry (8) could partially account for vascular localization of transplanted cells, a mere lack of control over the complex compositions of cell populations under investigation in certain studies could account for these controversies. Here, we show that although both ASC and AEC home to tumors, these different SVF populations independently engraft separate niches. Stromal/perivascular localization of ASC and vascular localization of AEC were observed, as well as partially preserved expression of pericyte and endothelial markers on ASC and AEC, respectively. Our study represents the first systematic comparative analysis of tumor homing and engraftment capacities displayed by different populations of WAT-derived cells. Although migration of WAT-derived macrophages to tumors was not analyzed in this study, it would be important to address in the future.

Bone marrow has been proposed as a source of mesenchymal tumor cells/CAF that might facilitate not only cancer progression but also cancer initiation (14, 48–50). Gene expression analyses have pointed to MSC as an origin of CAF (20, 22, 46, 51). As per the results presented here, recruitment of metronomically administered ASC (representing WAT MSC population) by tumors is sufficient to promote cancer progression. Recent studies in mouse tumor models show that systemically infused patient-derived adipose cells home to tumors (44) and that coadministration of mouse adipose cells with tumor cells promotes tumor take and growth (46, 47), consistent with our results. Because ASCs secrete angiogenic factors (30, 34), they could mediate cancer progression through stimulating tumor vascularization. The immunosuppressive effects of MSC (52) provide an additional possible mechanism for the ASC-dependent stimulation of tumor growth. Interestingly, several studies have reported lack of or inhibitory effects of MSC on cancer progression (21), consistent with stromal cells from bone marrow and lung being neutral in our studies. This could be explained either by unique functions inherent to stromal cells residing in different organs or by differential enrichment of stromal subpopulations with cancer-promoting properties in culture, which in our study might have occurred for WAT-derived but not for lung- and bone-derived MSC. The apparent heterogeneity of mesenchymal cells, reflected by heterogeneity of CD34 expression on stromal and perivascular cells depending on the organ and the vasculature type (28, 53), alerts for caution in making general conclusions about MSC functions without their rigorous comparative analysis (16). Although future studies will help to identify the predeterminants of stromal cell effect on tumors, a growing body of evidence suggests that ASC and bone marrow-derived MSC under certain conditions can accelerate cancer progression. These observations will require careful investigation of the effects of adult stem cell expansion *ex vivo* and most likely caution in their projected clinical applications in the context of cancer (35).

The uncovered role of ASC in supporting tumor neovascularization provides a new insight on the mechanisms linking obesity and cancer. Although our data do suggest that WAT provides a functional cellular contribution, soluble adipose factors are quite certainly implicated in tumor growth as well. WAT is a potent endocrine organ secreting hormones,

growth factors, and cytokines, such as insulin, leptin, adiponectin, hepatocyte growth factor, and insulin-like growth factor-I. These adipokines may stimulate cancer progression in addition to being integral components of the metabolic syndrome (6, 33, 36, 54). Given the stark contrast between experimental tumor models and true human cancer, follow-up clinical studies will be necessary to determine whether mobilization of adipose cells occurs in cancer patients and to define relative roles of cells and soluble factors and in disease progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Yach D, Stuckler D, Brownell KD. Epidemiologic and economic consequences of the global epidemics of obesity and diabetes. *Nat Med*. 2006; 12:62–6. [PubMed: 16397571]
2. Calle EE, Rodriguez C, Walker-Thurmond K, et al. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med*. 2003; 348:1625–38. [PubMed: 12711737]
3. Flegal KM, Graubard BI, Williamson DF, et al. Cause-specific excess deaths associated with underweight, overweight, and obesity. *JAMA*. 2007; 298:2028–37. [PubMed: 17986696]
4. Pierce J, Natarajan L, Caan BJ. Influence of a diet very high in vegetables, fruit, and fiber and low in fat on prognosis following treatment for breast cancer: the Women's Healthy Eating and Living (WHEL) randomized trial. *JAMA*. 2007; 298:289–98. [PubMed: 17635889]
5. Rehman J, Considine RV, Bovenkerk JE, et al. Obesity is associated with increased levels of circulating hepatocyte growth factor. *J Am Coll Cardiol*. 2003; 41:1408–13. [PubMed: 12706940]
6. Vona-Davis L, Howard-McNatt M, Rose DP. Adiposity, type 2 diabetes and the metabolic syndrome in breast cancer. *Obes Rev*. 2007; 8:395–408. [PubMed: 17716297]
7. Shaked Y, Ciarrocchi A, Franco M, et al. Therapy-induced acute recruitment of circulating endothelial progenitor cells to tumors. *Science*. 2006; 313:1785–7. [PubMed: 16990548]
8. Folkman J. Angiogenesis. *Annu Rev Med*. 2006; 57:1–18. [PubMed: 16409133]
9. Bertolini F, Shaked Y, Mancuso P, et al. The multifaceted circulating endothelial cell in cancer: towards marker and target identification. *Nat Rev Cancer*. 2006; 6:835–45. [PubMed: 17036040]
10. Kaplan RN, Riba RD, Zacharoulis S, et al. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. *Nature*. 2005; 438:820–7. [PubMed: 16341007]
11. Gao D, Nolan DJ, Mellick AS, et al. Endothelial progenitor cells control the angiogenic switch in mouse lung metastasis. *Science*. 2008; 319:195–8. [PubMed: 18187653]
12. Purhonen S, Palm J, Rossi D, et al. Bone marrow-derived circulating endothelial precursors do not contribute to vascular endothelium and are not needed for tumor growth. *Proc Natl Acad Sci U S A*. 2008; 105:6620–5. [PubMed: 18443294]
13. Peters BA, Diaz LA, Polyak K, et al. Contribution of bone marrow-derived endothelial cells to human tumor vasculature. *Nat Med*. 2005; 11:261–2. [PubMed: 15723071]
14. Wels J, Kaplan RN, Rafii S, et al. Migratory neighbors and distant invaders: tumor-associated niche cells. *Genes Dev*. 2008; 22:559–74. [PubMed: 18316475]
15. Du R, Lu KV, Petritsch C, et al. HIF1 α induces the recruitment of bone marrow-derived vascular modulatory cells to regulate tumor angiogenesis and invasion. *Cancer Cell*. 2008; 13:206–20. [PubMed: 18328425]
16. Bianco P, Robey PG, Simmons PJ. Mesenchymal stem cells: revisiting history, concepts, and assays. *Cell Stem Cell*. 2008; 2:313–9. [PubMed: 18397751]

17. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science*. 1997; 276:71–4. [PubMed: 9082988]
18. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999; 284:143–7. [PubMed: 10102814]
19. Nilsson SK, Simmons PJ. Transplantable stem cells: home to specific niches. *Curr Opin Hematol*. 2004; 11:102–6. [PubMed: 15257026]
20. Hall B, Dembinski J, Sasser AK, et al. Mesenchymal stem cells in cancer: tumor-associated fibroblasts and cell-based delivery vehicles. *Int J Hematol*. 2007; 86:8–16. [PubMed: 17675260]
21. Lazennec G, Jorgensen C. Concise review: adult multipotent stromal cells and cancer: risk or benefit? *Stem Cells*. 2008; 26:1387–94. [PubMed: 18388305]
22. Hu M, Yao J, Carroll DK, et al. Regulation of *in situ* to invasive breast carcinoma transition. *Cancer Cell*. 2008; 13:394–406. [PubMed: 18455123]
23. Aicher A, Rentsch M, Sasaki K, et al. Nonbone marrow-derived circulating progenitor cells contribute to postnatal neovascularization following tissue ischemia. *Circ Res*. 2007; 100:581–9. [PubMed: 17272807]
24. Grenier G, Scime A, Le Grand F, et al. Resident endothelial precursors in muscle, adipose, and dermis contribute to postnatal vasculogenesis. *Stem Cells*. 2007; 25:3101–10. [PubMed: 17823241]
25. Hausman DB, DiGirolamo M, Bartness TJ, et al. The biology of white adipocyte proliferation. *Obes Rev*. 2001; 2:239–54. [PubMed: 12119995]
26. Cartwright MJ, Tchkonian T, Kirkland JL. Aging in adipocytes: potential impact of inherent, depot-specific mechanisms. *Exp Gerontol*. 2007; 42:463–71. [PubMed: 17507194]
27. Spalding KL, Arner E, Westermark PO, et al. Dynamics of fat cell turnover in humans. *Nature*. 2008; 453:783–7. [PubMed: 18454136]
28. Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. *Circ Res*. 2007; 100:1249–60. [PubMed: 17495232]
29. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng*. 2001; 7:211–28. [PubMed: 11304456]
30. Traktuev D, Merfeld-Clauss S, Li J, et al. A population of multipotent CD34-positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks. *Circ Res*. 2008; 102:77–85. [PubMed: 17967785]
31. Rodeheffer MS, Birsoy K, Friedman JM. Identification of white adipocyte progenitor cells *in vivo*. *Cell*. 2008; 135:240–9. [PubMed: 18835024]
32. Tang W, Zeve D, Suh JM, et al. White fat progenitor cells reside in the adipose vasculature. *Science*. 2008; 322:583–6. [PubMed: 18801968]
33. Bell LN, Ward JL, Degawa-Yamauchi M, et al. Adipose tissue production of hepatocyte growth factor contributes to elevated serum HGF in obesity. *Am J Physiol Endocrinol Metab*. 2006; 291:E843–8. [PubMed: 16757549]
34. Rehman J, Traktuev D, Li J, et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation*. 2004; 109:1292–8. [PubMed: 14993122]
35. Kolonin MG, Simmons PJ. Combinatorial stem cell mobilization. *Nat Biotechnol*. 2009; 27:252–3. [PubMed: 19270674]
36. Baillargeon J, Rose DP. Obesity, adipokines, and prostate cancer. *Int J Oncol*. 2006; 28:737–45. [PubMed: 16465380]
37. Nunez NP, Perkins SN, Smith NC, et al. Obesity accelerates mouse mammary tumor growth in the absence of ovarian hormones. *Nutr Cancer*. 2008; 60:534–41. [PubMed: 18584488]
38. Hadjantonakis AK, Gertsenstein M, Ikawa M, et al. Generating green fluorescent mice by germline transmission of green fluorescent ES cells. *Mech Dev*. 1998; 76:79–90. [PubMed: 9867352]
39. Long JZ, Lackan CS, Hadjantonakis AK. Genetic and spectrally distinct *in vivo* imaging: embryonic stem cells and mice with widespread expression of a monomeric red fluorescent protein. *BMC Biotechnol*. 2005; 5:20. [PubMed: 15996270]

40. Jat PS, Noble MD, Ataliotis P, et al. Direct derivation of conditionally immortal cell lines from an H-2Kb-tsA58 transgenic mouse. *Proc Natl Acad Sci U S A*. 1991; 88:5096–100. [PubMed: 1711218]
41. Kolonin MG, Saha PK, Chan L, et al. Reversal of obesity by targeted ablation of adipose tissue. *Nat Med*. 2004; 10:625–32. [PubMed: 15133506]
42. Nie J, Chang B, Traktuev DO, et al. Combinatorial peptides identify $\alpha 5 \beta 1$ integrin as a receptor for the matricellular protein SPARC on adipose stromal cells. *Stem Cells*. 2008; 26:2735–45. [PubMed: 18583538]
43. Klebanov S, Astle CM, DeSimone O, et al. Adipose tissue transplantation protects ob/ob mice from obesity, normalizes insulin sensitivity and restores fertility. *J Endocrinol*. 2005; 186:203–11. [PubMed: 16002549]
44. Kucerova L, Altanerova V, Matuskova M, et al. Adipose tissue-derived human mesenchymal stem cells mediated prodrug cancer gene therapy. *Cancer Res*. 2007; 67:6304–13. [PubMed: 17616689]
45. Grove JE, Bruscia E, Krause DS. Plasticity of bone marrow-derived stem cells. *Stem Cells*. 2004; 22:487–500. [PubMed: 15277695]
46. Galie M, Konstantinidou G, Peroni D, et al. Mesenchymal stem cells share molecular signature with mesenchymal tumor cells and favor early tumor growth in syngeneic mice. *Oncogene*. 2007; 27:2542–5. [PubMed: 17998939]
47. Muehlberg F, Song YH, Krohn A, et al. Tissue resident stem cells promote breast cancer growth and metastasis. *Carcinogenesis*. 2009; 30:589–97. [PubMed: 19181699]
48. Bhowmick NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. *Nature*. 2004; 432:332–7. [PubMed: 15549095]
49. Direkze NC, Hodivala-Dilke K, Jeffery R, et al. Bone marrow contribution to tumor-associated myofibroblasts and fibroblasts. *Cancer Res*. 2004; 64:8492–5. [PubMed: 15574751]
50. Karnoub AE, Dash AB, Vo AP, et al. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature*. 2007; 449:557–63. [PubMed: 17914389]
51. Udagawa T, Puder M, Wood M, et al. Analysis of tumor-associated stromal cells using SCID GFP transgenic mice: contribution of local and bone marrow-derived host cells. *FASEB J*. 2006; 20:95–102. [PubMed: 16394272]
52. Jones BJ, McTaggart SJ. Immunosuppression by mesenchymal stromal cells: from culture to clinic. *Exp Hematol*. 2008; 36:733–41. [PubMed: 18474304]
53. Crisan M, Yap S, Casteilla L, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell*. 2008; 3:301–13. [PubMed: 18786417]
54. Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. *Br J Nutr*. 2004; 92:347–55. [PubMed: 15469638]

Figure 1.

Adipose SVF cells home to tumors. *A*, 10^6 GFP⁺ SVF cells were i.v. injected into BALB/c mice carrying EF43. *fgf4* tumors and let circulate for 1 d. *B* to *D*, 10^6 GFP⁺ SVF cells were i.v. injected into nude mice carrying MDA-231 (*B*) or KS1767 (*C* and *D*) xenografts and let to circulate for 7 d. In *A* to *D*, sections of tumors and control tissues were subjected to anti-GFP immunofluorescence (green arrows). Red counterstaining was performed with anti-CD31 antibodies (*B* and *C*) or anti-Ki67 antibodies (*D*)/secondary Cy3-conjugated antibodies (red), which were also used without the primary antibody to set the autofluorescence background (*A*). In *B* and *C*, DNA is stained blue with TOPRO3. *D*, inset, high magnification, reveals coexpression of GFP and Ki67. Z-stacks reveal GFP⁺ multinucleate (*C*, blue arrows) and proliferating (*D*, red arrows) cells. Scale bar, 100 μm.

Figure 2.

Adipose SVF cells incorporate into tumor stroma and vasculature. GFP⁺ SVF cells (10^6) were i.v. injected into mice carrying KS1767 xenografts and let to circulate for 14 d. Tumor sections were subjected to anti-GFP (green) and anti-CD31 (A, red) or anti-αSMA (B, red) confocal immunofluorescence. Yellow signal on digital channel merging (right) reveals vascular donor GFP⁺/CD31⁺ cells (A) and perivascular GFP⁺/αSMA⁺ cells (B). Insets, high magnification of selected areas. Nuclear Hoechst 33258 staining (blue) confirms cell being double-positive for GFP and CD31. Scale bar, 50 μm.

Figure 3.

Tumor homing and engraftment of ASC and AEC. *A*, separation of freshly isolated GFP⁺ SVF cells into CD45⁻CD34⁺CD31⁻ (ASC) and CD45⁻CD34⁺CD31⁺ (AEC). *B*, cultures of ASC (CD31⁻) and AEC (CD31⁺) populations. *C*, two representative anti-GFP (green)/anti-CD31 (red) confocal immunofluorescence images of KS1767 xenografts from mice injected with 2.5×10^5 of ASC (left) or AEC (right) 14 d before tumor recovery. Notice ASC being CD31⁻ and some AEC being CD31⁺ (overlapping yellow signal). Z-stack projection of a median series through the TOPRO3⁺ (blue) nucleus confirms single-cell identity of GFP⁺CD31⁺ AEC. Bottom, separated GFP and CD31 channels. Scale bar, 10 μ m.

Figure 4.

S.c.-injected ASCs home to tumors via systemic circulation. Biodistribution of 10^6 GFP⁺ SVF cells in mice carrying KS1767 xenografts after administration i.v., s.c. proximately to the tumor, and s.c. distantly to the tumor. *A*, quantification of GFP⁺ cells in PBMC 360 min after distant s.c. injection by flow cytometry on PBMC compared with PBMC from an untreated mouse. *B*, time course of GFP⁺ cell circulation showing that the majority of adipose cells are present in the bloodstream at 15 to 360 min after s.c. injection. *C*, *green*, tumor sections 14 d after injection were subjected to anti-GFP immunofluorescence. Scale bar, 50 μ m.

Figure 5.

Tumor growth is promoted by ASC. Mice xenografted (at week 0) with KS1767 or DU145 tumors have been s.c. injected (lower back) starting 1 d after xenografting for 6 wk with 10^4 of the following mouse cells per day: Immorto/GFP ASC ($n = 10$), Immorto/GFP lung stromal cells ($n = 5$), Immorto/GFP bone stromal cells ($n = 5$), 3T3-L1 fibroblasts ($n = 5$), PBS ($n = 5$), or were noninjected ($n = 5$). **A**, tumor growth in mice injected with ASC compared with the indicated controls. Measured over time is the average fold increase of tumor size over the size measured at week 2. Points, mean; bars, SE. *, $P < 0.05$ (Student's t test). **B**, representative ASC-treated and untreated mice at week 4. **C**, sections of tumors from representative ASC-treated mice at week 6 subjected to anti-GFP (green)/anti-CD31 (red) confocal immunofluorescence. Blue, nuclear TOPRO3 staining. Scale bar, 20 μm .

Figure 6.

Cell recruitment from WAT is associated with tumor growth. *A*, representative mouse with a WAT implant (green arrow) 10 d after implantation. *Right*, section of WAT implant 30 d after implantation. *B*, growth of KS1767 and LLC tumors in mice with (green) or without (white) WAT implants. Points, mean between five mice per group; bars, SE. *, $P < 0.1$ (KS1767) and $P < 0.05$ (LLC; Student's test). *C*, representative WAT-implanted and control mice at week 2. *D*, section of a KS1767 tumor from a WAT-implanted mouse subjected to anti-GFP (green)/anti-CD31 (red) immunofluorescence. Blue, nuclear Hoechst 33258 staining. Scale bar, 100 μ m.