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RESEARCH ARTICLE

Human adipose tissue from normal and tumoral breast regulates the behavior of mammary epithelial cells

Virginia Pistone Creydt · Sabrina Johanna Fletcher · Jimena Giudice · Ariana Bruzzone · Norma Alejandra Chasseing · Eduardo Gustavo Gonzalez · Paula Alejandra Sacca · Juan Carlos Calvo

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Abstract
Introduction Stromal-epithelial interactions mediate both breast development and breast cancer progression. In the present work, we evaluated the effects of conditioned media (CMs) of human adipose tissue explants from normal (hATN) and tumor (hATT) breast on proliferation, adhesion, migration and metalloproteases activity on tumor (MCF-7 and IBH-7) and non-tumor (MCF-10A) human breast epithelial cell lines.
Materials and methods Human adipose tissues were obtained from patients and the conditioned medium from hATN and hATT collected after 24 h of incubation. MCF-10A, MCF-7 and IBH-7 cells were grown and incubated with CMs and proliferation and adhesion, as well as migration ability and metalloprotease activity, of epithelial cells after exposing cell cultures to hATN- or hATT-CMs were quantified. The statistical significance between different experimental conditions was evaluated by one-way ANOVA. Tukey′s post hoc tests were performed.
Results Tumor and non-tumor breast epithelial cells significantly increased their proliferation activity after 24 h of treatment with hATT-CMs compared to control-CMs. Furthermore, cellular adhesion of these two tumor cell lines was significantly lower with hATT-CMs than with hATN-CMs. Therefore, hATT-CMs seem to induce significantly lower expression or less activity of the components involved in cellular adhesion than hATN-CMs. In addition, hATT-CMs induced pro-MMP-9 and MMP-9 activity and increased the migration of MCF-7 and IBH-7 cells compared to hATN-CMs.
Conclusions We conclude that the microenvironment of the tumor interacts in a dynamic way with the mutated epithelium. This evidence leads to the possibility to modify the tumor behavior/phenotype through the regulation or modification of its microenvironment. We developed a model in which we obtained CMs from adipose tissue explants completely, either from normal or tumor breast. In this way, we studied the contribution of soluble factors independently of the possible effects of direct cell contact.

Keywords Human breast adipose tissue · Breast epithelial cells · Cancer · Epithelial-stromal interactions

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Introduction

The mammary epithelium is surrounded by multiple types of stromal cells, including preadipocytes, adipocytes, fibroblasts, vasculature, pericytes, and macrophages [1]. Adipocytes are the predominant stromal cell type in the breast tissue microenvironment [2]. Stromal-epithelial interactions mediate breast development and breast cancer progression [3]. Adipose tissue is an endocrine organ [4] that secretes soluble factors such as interleukins (IL-6, IL-

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1β) [5–7], growth factors [IGF1, VEGF and hepatocyte growth factor (HGF)] [5, 8, 9] and adipokines (leptin and adiponectin) [9]. Thus, it significantly contributes to the composition of the extracellular matrix (ECM). Recent studies have shown that the IL-6 secreted from adipose stromal cells promotes migration and invasion of breast cancer cells [7]. In addition, cancer-associated adipocytes express abnormal levels of IGF1, leptin and adiponectin and leptin appears to be a positive factor for tumor development [5, 9]. Other soluble factors, such as HGF, may act not only to promote tumor cell invasion, but also to enhance tumor growth indirectly by stimulating angiogenesis [9]. Razmkhah et al. [10] have recently reported that resident adipose-derived stem cells (hASCs) in breast cancer tissue may have crucial roles in breast tumor growth and progression by promoting anti-inflammatory reaction within the tumor microenvironment through the expression of IL-4, IL-10 and TGF-β1.

Breast cancer is a common cause of cancer-related death among women [11]. Metastasis is a multistage process that requires cancer cells to escape from the primary tumor, survive in the circulation, seed at distant sites and grow. Each of these processes involves rate-limiting steps that are influenced by non-malignant cells of the tumor microenvironment [12]. The invasion of tumor cells into healthy tissues is promoted by local proteolysis of the ECM. This process involves metalloproteases (MMPs), which are members of an endopeptidase family, which cleaves ECM components, as well as different cell modulators secreted by or present on the cell surface [13]. The MMP-2 and MMP-9 gelatinases play a key role in the proteolytic cascade of matrix degradation during invasion and metastasis. An increase in MMP-2 and MMP-9 in human breast tumors has been reported and associated with a poor prognosis. We have recently shown that incubation of LM3 cells (murine mammary tumor epithelial cells) with conditioned medium from the 3T3-L1 cell line (murine preadipocyte cells) at different stages of differentiation stimulates the expression of MMP-9 [14].

In the last decade, different reports have shown that the stroma is a crucial target of carcinogenesis [15] and that the neoplastic phenotype is context-dependent. Elucidation of these mechanisms could contribute to cancer reversal and cure [16]. When trying to understand the genesis of cancer, the classical view is the somatic mutation theory which explains cancer as the consequence of an accumulation of mutations and other heritable changes in susceptible cells. More recently, an alternative paradigm, the tissue organization field theory, proposes cancer as a tissue-based disease [17]. However, it is still necessary to explore the role of specific stromal components (fibroblasts, adipocytes, mast cells, etc.,) and the ECM in mammary carcinogenesis. Some studies have evaluated cell interactions between adipocytes and normal and tumor mammary epithelium [18, 19]. However, the ability of adipose tissue to modify the biological behavior of breast epithelial cells is not well known. Pinilla et al. [20] have recently investigated whether human adipose tissue-derived stem cells (hASCs) enhance tumor invasion being a potential source of CCL5, a chemokine involved in tumor progression. In addition, Dirat et al. [6, 21] have studied the role of IL-6 secreted by mammary adipocytes and by the adipose cell line 3T3-F442A on the behavior of mammary tumor cell lines.

We have previously shown that soluble and insoluble factors present at different stages of differentiation of the 3T3-L1 cell line, differentially regulate growth and migration of normal (NMuMG) and tumor (LM3) murine mammary epithelial cells [14].

The use of freshly excised human adipose tissue explants, although more tedious to create, makes the conditions of an experiment closer to reality [22–24]. Here, we investigated the effect of conditioned media (CMs) of human adipose tissue from normal (hATN) and tumor (hATT) breast on proliferation, adhesion, migration and activity of MMPs on tumor (MCF-7 and IBH-7) and non-tumor (MCF-10A) human breast epithelial cell lines. We developed a model in which we obtained CMs from adipose tissue explants completely, from both normal and tumor breast. In this way, we studied the contribution of soluble factors independently of the possible effects of direct cell contact.

Materials and methods

Reagents

Reagents were from Sigma Chemical Co (St. Louis, MO, USA), tissue culture flasks, dishes, and multi-well plates were from Falcon Orange Scientific (Graignette Business Park, Belgium), and culture media from both tissue and cell lines and supplements were from Gibco BRL (Carlsbad, CA, USA).

Sample collection and handling

Human adipose tissue from normal (hATN; $n = 6$) and tumor (hATT; $n = 9$) breast were obtained from patients that underwent surgery procedure for esthetic purposes (normal breast) or radical mastectomy (tumor breast), who had not received previous chemotherapy or radiotherapy treatment. Tumors from hATT samples were infiltrating ductal tumors (stage GH2). All tumors were estrogen and progesterone receptor positive. Samples were transported in PBS with gentamicin (50 µg/ml) and processed immediately. On average, 2 h elapsed from the acquisition of the

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surgical sample until it was processed under a sterile laminar flow hood. The project was approved by the ethics committee of the Instituto de Biología y Medicina Experimental (IByME), Argentina and all patients gave their informed consent to undergo tissue harvesting for this research.

Preparation of conditioned media (CMs) from hATN and hATT

Adipose tissues were washed three times with cold PBS to remove red blood cells and debris, and weighed. hATN or hATT were plated in a culture flask with M199 culture medium (Invitrogen™; 1 g tissue/10 ml M199) supplemented with gentamicin (50 µg/ml) and incubated for 1 h at 37 °C in 5 % CO_2. After that, the medium was removed and replaced with fresh medium and the tissues were incubated for 24 h. Subsequently, the supernatants were collected and the cells were removed by centrifugation (3 min at $400 \times g$). Then, the supernatants were aliquoted into 1-ml fractions and immediately stored at −80 °C. The control-CMs were obtained from the collection of serum free M199 medium after 24 h of incubation in a culture flask at 37 °C in 5 % CO_2.

Treatment with hATN or hATT-CMs

In order to study proliferation, migration and activity of MMPs, MCs collected were diluted 1:1 in DMEM-F12 (Invitrogen, UK) 2 % fetal bovine serum (FBS; 1 % FBS final concentration) and the cells were incubated with the diluted CMs. The experiments were performed with equal volumes of hATT- and hATN-CMs. The concentration of total protein in those volumes was quantified using Bradford reagent.

Culture of tumor and non-tumor breast epithelial cell lines

Tumor (MCF-7 and IBH-7) and non-tumor (MCF-10A) human breast epithelial immortalized cell lines were used. MCF-7 and MCF-10A were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA), whereas IBH-7 is a line immortalized from a primary breast tumor by the laboratory of Dr. Isabel Luthy [25, 26]. MCF-7 and IBH-7 cells were cultured in DMEM-F12 medium with 10 % FBS and 2 µg/ml insulin. MCF-7 and IBH-7 are estrogen receptor-positive cell lines. MCF-10A cells were cultured in DMEM-F12 medium supplemented with 10 % FBS, 2 µg/ml insulin, 0.5 µg/ml cortisol and 20 ng/ml EGF. The three cell lines were maintained at 37 °C in 5 % CO_2.

Preparation of CMs from breast epithelial cells after incubation with hATN-CMs or hATT-CMs

MCF-10A, MCF-7 and IBH-7 cells were seeded in six-well plates in DMEM-F12 complete medium. When cells reached 75–80 % confluence, the medium was aspirated and cells were washed twice with PBS. Then, cells were incubated at 37 °C for 48 h either with or without hATN or hATT-CMs. The resulting CMs of MCF-10A and IBH-7 were clarified by centrifugation at 4 °C and stored at −80 °C.

Breast epithelial cell proliferation assay

Tumor (3×10^3 MCF-7 or IBH-7 cells/well) and non-tumor (5×10^3 MCF-10A cells/well) human breast epithelial cell lines were incubated on 96-well plates with complete DMEM-F12 for 24 h. Then, the three cell lines were treated with hATN-, hATT- or control-CMs for an additional 24 h. The number of viable cells was determined by a commercial colorimetric kit (Cell titer 96 AQueous One Solution Cell Proliferation Assay, MTS). Results are expressed as percentage of color intensity and normalized to cells grown in control-CMs. In addition, we evaluated cell proliferation by incorporation of (^{3}H)-thymidine.

Cellular adhesion assays

Adhesion assays were performed following a protocol previously reported [27]. Briefly, 96-well plates were coated with 100 µl hATN-, hATT- or control-CMs at 37 °C overnight in 5 % CO_2. These CMs were set in three wells and each experiment was repeated three times. Plates were then blocked with 1 mg/ml bovine serum albumin at 37 °C for 1 h. After washing with PBS, MCF-10A, MCF-7 or IBH-7 cells (5×10^4 cells/well) were suspended in serum-free DMEM-F12 medium, seeded and allowed to adhere to the CMs factors-coated wells at 37 °C for 1 h in 5 % CO_2. Non-adherent cells were aspirated and wells washed twice with PBS. Residual cells were evaluated by MTS assays. Cell adhesion to hATN- or hATT-CMs factors was expressed as percentage of control-CMs.

Breast epithelial cell migration assays

The effect of hATN- and hATT-CMs on the motility of tumor breast epithelial cell lines was evaluated by wound-healing and by Transwells migration assays

Wound-healing assays

MCF-10A, MCF-7 and IBH-7 cells were grown on 96-well plates with complete DMEM-F12 for 24 h and then treated with hATN-, hATT- or control-CMs for 24 h. After that,

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cells were wounded with a 200 μl pipette tip, washed twice with PBS and hATN-, hATT- or control-CMs were added. Images at zero time (0 h) were captured to record the initial width of the wounds. The recovery of the wounded monolayers due to cell migration toward the denuded area was evaluated after 6 h. The images were acquired by an inverted phase-contrast microscope (Olympus CKX-41) using a 4× objective. Quantification was performed using ImageJ (NIH, Bethesda, MD, USA) by a polygon selection mode and determining the percentage of the wounded area at 6 h respect to control (0 h)

Transwells migration assays

MCF-10A, MCF-7 and IBH-7 cells (2×10^5 cells/0.2 ml) were placed into the top transwell with 8 μm pore membranes (NUNC cat.#140629). They were then incubated with hATN-, hATT- or control-CMs and allowed to transmigrate across the porous membrane for 20 h. At the end of the assay, inserts were removed and the cells were fixed in 4 % paraformaldehyde and then stained with a 0.1 % crystal-violet solution. Tumor and non-tumor cells on the upper membrane surface were removed with a paper towel. The air-dried membranes were viewed under 20× magnification and migrated cells were counted in 5 randomly chosen fields per membrane.

Metalloprotease activity assays

Mono-dimensional gelatin zymography was performed under non-reducing conditions on 7.5 % (w/v) gel copolymerized with 1 mg/ml gelatin to identify proteins with gelatinolytic activities. We analyzed CM aliquots of 30 μl (in triplicate) from hATN, hATT, MCF-10A, MCF-10A-hATN, MCF-10A-hATT, MCF-7, MCF-7-hATN and MCF-7-hATT.

After the electrophoresis, the SDS was removed from the gels by two washes with 2.5 % Triton-X 100 in PBS. Then, the gels were incubated at 37 °C for 22 h in an activating buffer (50 mM Tris–HCl, pH 7.5, 200 mM NaCl, 5 mM $CaCl_2$), and then stained with 0.5 % (w/v) Coomassie Brilliant Blue R-250. Proteolytic activities were visualized as unstained areas after destaining the gel with 7 % methanol/5 % acetic acid, indicating areas where gelatin was degraded by the enzymes. The area of the gelatinolytic bands was evaluated by densitometry analysis by Scion Corp software. Molecular weights of gelatinolytic bands were estimated by comparing their electrophoretic migration to that of standard proteins (BioRad Laboratories, Inc., CA, USA). To analyze metalloproteinase inhibition, the gels were incubated with 20 mM EDTA.

Statistical analysis

The statistical significance between different experimental conditions was evaluated by one-way ANOVA. Tukey's post hoc tests were performed within each individual treatment. The results are presented as mean ± SEM. Results were considered significant at $p < 0.05$.

Results

The hATT-CMs increase the proliferation of tumor and non-tumor mammary epithelial cells

To evaluate the effect of hATN- or hATT-CMs on the proliferation of tumor (MCF-7 and IBH-7) and non-tumor (MCF-10A) epithelial cells, the three cell lines were incubated for 24 h with hATN-, hATT- or control-CMs. The total amount of protein was quantified in the different CMs: hATN-CMs: 1.12 ± 0.26 μg/μl ($n = 6$), and hATT-CMs: 0.18 ± 0.08 μg/μl ($n = 9$).

We then evaluated proliferation either by the MTS technique (Fig. 1a) or by the (^{3}H)-thymidine incorporation assay (Fig. 1b), and found consistent results. Data

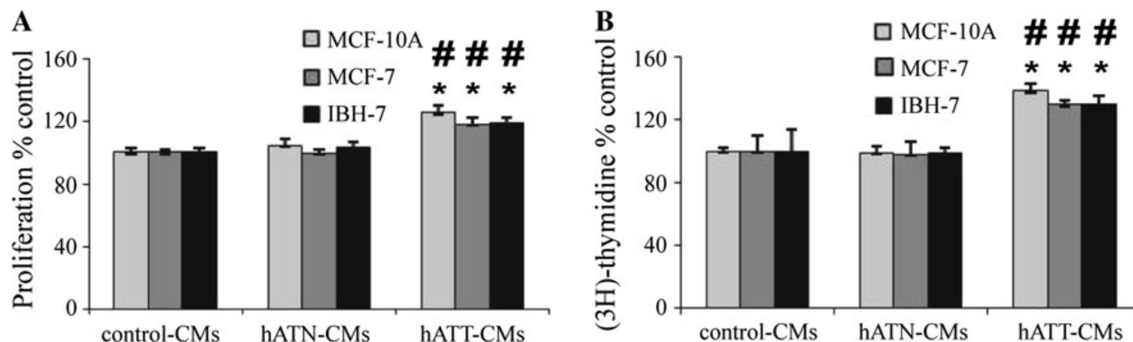

Fig. 1 Effect of CMs from hATN and hATT on proliferation of MCF-10A, MCF-7 and IBH-7 cells. MCF-10A, MCF-7 and IBH-7 cells were incubated with hATN- ($n = 6$), hATT- ($n = 9$) or control-CMs for 24 h. Proliferation was measured by MTS assays (**a**) or by incorporation of (^{3}H)-thymidine (**b**). Data are shown as the mean ± SEM ($n = 5$–7 experiments by triplicate). $^*p < 0.01$ hATT-CMs versus hATN-CMs; $^\#p < 0.01$ ATT-CMs versus control-CMs

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showed that hATT-CMs significantly increased the proliferation activity compared to control- and hATN-CMs after 24 h ($p < 0.01$), and that hATN-CMs did not modify the proliferation ability of any of the three cell lines compared to control-CMs after 24 h incubation with CMs (Fig. 1).

These results suggest the presence of soluble factors in the hATT-CMs (absent in the hATN-CMs) that would stimulate the proliferation of both tumor and non-tumor epithelial cells. Another possibility could be the presence of inhibitory soluble factors in the hATN-CMs that are absent in the hATT-CMs. The characterization of soluble factors in both CMs will help to elucidate this question.

The hATT-CMs decrease the adhesion of tumor mammary epithelial cells

In order to compare the content of soluble factors present in hATN- and hATT-CMs that could be involved in tumor cell adhesion, we performed adhesion experiments. MCF-10A, MCF-7 and IBH-7 cells were seeded in plates previously exposed to different CMs. hATT-CMs significantly decreased the adhesion capacity of MCF-7 and IBH-7 cells with respect to a surface covered with hATN-CMs ($p < 0.001$) (Fig. 2). In contrast, hATN-CMs increased MCF-7 and IBH-7 cell adhesion compared with that in control-CMs ($p < 0.001$) (Fig. 2). On the other hand, hATN- and hATT-CMs did not affect MCF-10A cell adhesion compared to control-CMs (Fig. 2).

This indicates that the hATT-CMs contain fewer factors involved in tumor mammary epithelial cell adhesion than hATN-CMs. The decrease in adhesion would allow tumor cells to migrate and colonize new target organs in vivo.

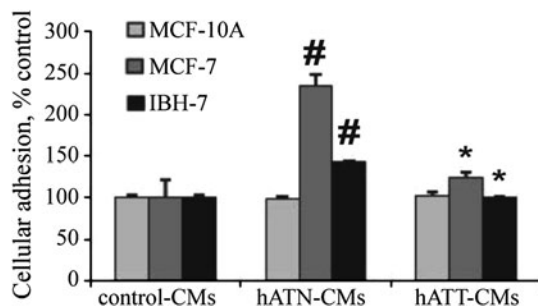

Fig. 2 Effect of CMs from hATN and hATT on MCF-10A, MCF-7 and IBH-7 cell attachment. MCF-10A, MCF-7 and IBH-7 were plated at a density of 5×10^4 cells/well in wells preincubated with hATN- ($n = 3$–6), hATT- ($n = 5$–9) or control-CMs by 12 h and adherent cells were quantified by MTS. Data are shown as the mean ± SEM ($n = 3$ experiments by triplicate). $^*p < 0.001$ hATT-CMs versus hATN-CMs and $^{\#}p < 0.001$ hATN-CMs versus control

Migration of tumor mammary epithelial cells increases with hATT-CMs incubation

To evaluate the effect of hATN- and hATT-CMs on the migratory capacity of tumor and non-tumor mammary epithelial cells, we performed wound healing and Transwells migration assays. Figure 3a–d shows that hATT-CMs significantly increased the migration of MCF-7 and IBH-7 tumor cells, after 6 h of incubation, compared to the effect of hATN-CMs ($p < 0.01$) and control-CMs ($p < 0.01$). Moreover, MCF-7 and IBH-7 cells did not show significant changes in cell motility after 6 h when they were incubated with hATN-CMs compared to control-CMs. We obtained similar results by Transwells migration assays (Fig. 3e), i.e. hATT-CMs significantly increased the transmigration of MCF-7 and IBH-7 tumor cells across the porous membrane, compared to the effect of hATN-CMs ($p < 0.05$) and control-CMs ($p < 0.05$).

MCF-10A cells did not show significant changes in cell motility when they were incubated with control-, hATN- or hATT-CMs.

These results suggest that hATN-CMs increase the migration capacity of tumor mammary epithelial cells.

The hATN-CMs show pro-MMP-9 and MMP-9 activity

MMP-2 and MMP-9 belong to the gelatinases subtype and participate in ECM degradation during invasion and metastasis. Gelatinolytic activity was measured by zymography in hATN- and hATT-CMs, and in the media collected from MCF-10A and MCF-7 cell cultures treated with different MCs for 24 h. Figure 4 shows representative zymographic analysis. Bands of approximately 92 kDa corresponded to standards of latent pro-MMP-9 and bands of approximately 88 kDa corresponded to standards of MMP-9. Gelatinolytic bands of pro-MMP-9 and MMP-9 were observed in hATT-CMs: pro-MMP-9 activity, 1,521 ± 311 and MMP-9 activity, 1,800 ± 593 (Fig. 4a, c; lanes 1, 2, 5, 6). In contrast, hATN-CMs (Fig. 4a, lanes 3, 4 and 7) and control-CMs (Fig. 4a, lane 8) showed no basal activity of pro-MMP-9 or MMP-9.

None of the CMs from MCF-10A and MCF-7 cells showed basal activity of pro-MMP-9 or MMP-9. Furthermore, incubation of cell lines with hATN- or hATT-CMs showed no significant changes in the gelatinolytic activity of pro-MMP-9 or MMP-9 (Fig. 4b, c).

No gelatinolytic bands were detectable when the gels were incubated with 20 mM EDTA, a typical inhibitor of MMPs (data not shown).

Taken together, these results suggest that adipose tissue of breast tumors secrete higher levels of pro-MMP-9 and MMP-9 than adipose tissue of normal breast.

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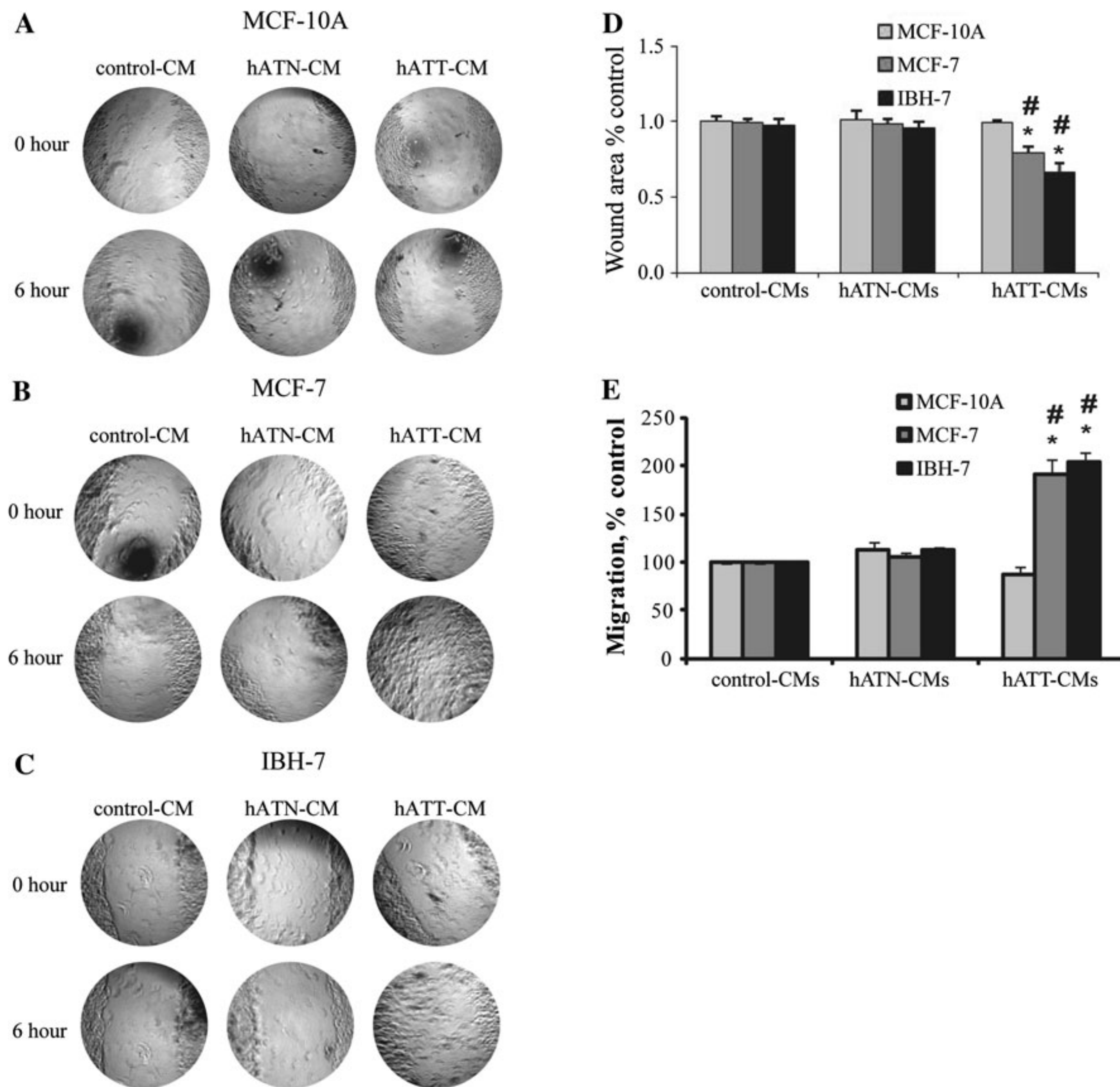

Fig. 3 Effect of CMs from hATN and hATT on migration of MCF-10A, MCF-7 and IBH-7 cells. *Wound healing assay* (**a–d**). MCF-10A, MCF-7 and IBH-7 cells were grown, incubated with hATN-, hATT- or control-CMs by additional 24 h. After that, cells were wounded, washed twice with PBS and hATN- ($n = 4$), hATT- ($n = 8$) or control-CMs were added. Images were captured at the wound instant and after 6 h. We show representative light microscopic images (4×). **a** MCF-10A, **b** MCF-7 or **c** IBH-7 incubated with hATN-, hATT or control-CMs. **d** The *histogram* shows the ratio of 6 h/0 h cutting area and was *plotted* as mean ± SEM ($n = 3$ experiments by duplicate). $^*p < 0.01$ hATT-CMs versus hATN-CMs; $^\#p < 0.01$ hATT-CMs versus control-CMs. *Transmigration assay* **e** MCF-10A, MCF-7 and IBH-7 cells were incubated with hATN-, hATT- or control-CMs and allowed to migrate across the porous membrane for 20 h. The membranes were viewed under 20× magnification and migrated cells were counted in 5 randomly chosen fields per membrane. Data are shown as the mean ± SEM ($n = 3$ experiments by duplicate). $^*p < 0.05$ hATT-CMs versus hATN-CMs and $\#p < 0.05$ hATT-CMs versus control-CMs

Discussion

Stromal-epithelial interactions mediate breast development and breast cancer progression. Adipocytes are the predominant stromal cell type present in breast tissue. The aim of this work was to study the importance of the adipose tissue microenvironment in the regulation of the biological behavior of breast epithelial cells. To this end, we evaluated the effects of hATT- and hATN-CMs from patients with or without breast cancer on cell proliferation, adhesion, migration and metalloprotease activity of MCF-10A, MCF-7 and IBH-7 cells. All hATT samples were from estrogen and progesterone receptor-positive ductal breast tumors, and both MCF-7 and IBH-7 cells were estrogen receptor-positive.

Taken together, our evidence shows that the adipose tissue surrounding a tumor regulates tumor epithelial cell adhesion to stroma, collaborating in the ability of breast epithelial cells to colonize new organs. Our results suggest that CMs of human adipose tissue modulate proliferation,

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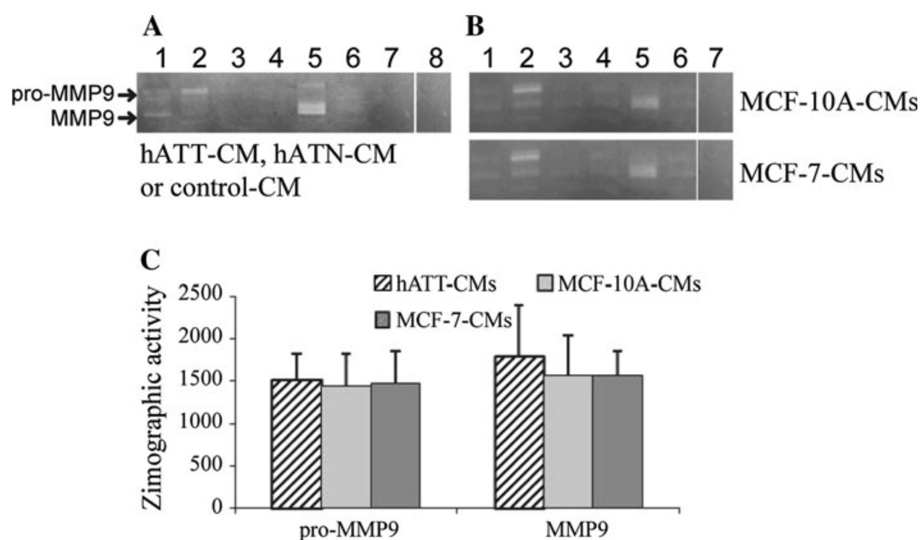

Fig. 4 Measurements of gelatinolytic activity. **a** Representative gelatin zymograms of hATN-, hATT- or control CMs. hATT-CM: *lanes 1*, *2*, *5* and *6*; hATN-CM: *lanes 3*, *4* and *7*; control-CM: *lane 8*. **b** Representative gelatin zymograms of MCF-10A- and MCF-7-CMs incubated with hATN-CMs (*lanes 3*, *4* and *6*) and hATT-CMs (1, 2 and 5). *Lane 7* shows basal activity of cells. The reaction was positive for bands of approximately 92 kDa corresponding to pro-MMP-9 and bands of approximately 88 kDa corresponding to MMP-9. The area of the zymographic activity was evaluated by densitometry analysis. The *histogram* shows the mean ± SD of three independent experiments (**c**)

adhesion and migration of tumor and non-tumor breast cancer cells. The effects observed are stronger if we consider that we worked with equal volumes of hATT- and hATN-CMs, and that the amount of total protein present in hATT-CMs is one order of magnitude less than the total protein present in hATN-CMs.

The cellular microenvironment is a complex system, with potential contributions of both physical adhesion and soluble factors. The model used in the present work did not allow studying the possible contributions of physical cell contact. Nevertheless, it did allow obtaining complete CMs from adipose tissue explants, both from normal and tumor breast. In this way, we studied the contribution of soluble factors independently of the possible effects of direct cell contact.

The present results allow viewing cancer, which is focused only on oncogenes and tumor suppressor genes, from a new point of view where the tumor microenvironment co-evolves and interacts in a dynamic and reciprocal way with the mutated epithelium [5, 28, 29] and where, consequently, tumor behavior/phenotype could be modified through the regulation or modification of the microenvironment.

Further experiments are needed to allow the identification of components that play a role in the paracrine interaction between tumor or normal breast cells and the surrounding stroma. To perform this characterization, we will use CMs obtained from samples used in the present work, as well as CMs from other samples recently obtained. These new samples are being tested for their ability to stimulate proliferation, adhesion and migration of the different epithelial cell lines. We are presently using growth factor and cytokine arrays commercially available to establish a proteomic profile of the conditioned medium released from explants.

In addition, by digestion of human adipose tissue explants, we are presently developing primary cultures of preadipocytes and adipose-stem cells (ASCs), and obtaining mature adipocytes by differentiation of the primary cultures. Preliminary results allow us to establish that preadipocytes and mature adipocytes differentially modulate the growth of mammary epithelial cells both through soluble factors (CMs) and direct cell–cell contact (stromal support) [30]. Therefore, we are beginning to elucidate the possible factors involved in the results obtained, as well as to identify the role played by different components in the synthesis and release of these factors. Characterization of these factors could potentially provide new strategies to regulate the development of normal breast tissue and/or prevent and treat breast cancer.

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Conflict of interest None.

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