Promoting effects of adipose-derived stem cells on breast cancer cells are reversed by radiation therapy

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Abstract Partial breast irradiation of early breast cancer patients after lumpectomy and the use of endogenous adipose tissue (AT) for breast reconstruction are promising applications to reduce the side effects of breast cancer therapy. This study tries to investigate the possible risks associated with these therapeutic approaches. It also examines the influence of adipose derived stem cells (ADSCs) as part of the breast cancer microenvironment, and endogenous AT on breast cancer cells following radiation therapy. ADSCs, isolated from human reduction mammoplasties of healthy female donors, exhibited multilineage

capacity and specific surface markers. The promoting effects of ADSCs on the growth and survival fraction of breast cancer cells were reversed by treatment with high (8 Gy) or medium (2 Gy) radiation doses. In addition, a suppressing influence on breast cancer growth could be detected by co-culturing with irradiated ADSCs (8 Gy). Furthermore the clonogenic survival of unirradiated tumor cells was reduced by medium of irradiated ADSCs. In conclusion, radiation therapy changed the interactions of ADSCs and breast cancer cells. On the basis of our work, the importance of further studies to exclude potential risks of ADSCs in regenerative applications and radiotherapy has been emphasized.

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Abbreviations

7-AAD	7-Aminoactinomycin
ADSCs	Adipose-derived stem cells
AT	Adipose Tissue
BrdU	Bromodeoxyuridine
CM	Conditioned medium
DMEM-F12	Dulbecco's modified Eagle medium and Kaighn's modification of Ham's F12
DMSO	Dimethyl sulfoxide
FBS	Fetal bovine serum Superior
MT	Medium transfer

NSABP	National Surgical Adjuvant Breast and Bowel Project
P/S	Penicillin/streptomycin
PBS	Phosphate-buffered saline
PE	Plating efficiency
RIBEs	Radiation-induced bystander effects
SD	Standard deviation
SEM	Standard error of mean
SF	Survival fraction

Introduction

Breast cancer is the most common cancer in women worldwide with approximately 1.7 million new cases diagnosed in 2012 (Ferlay et al. 2015). Surgery is the standard therapy in breast cancer. Surgical options depend on the stage of the disease. These range from lumpectomy, the removal of the cancer and minimal surrounding tissue, a breast-conserving surgery in early-stage breast cancer patients or alternatively for more advanced stages of the disease, whole breast removal (mastectomy). For reconstruction of the breast, implants are still used. However, lipografting with endogenous AT appears to be a promising option for the future (reviewed in Gir et al. 2012). An advantage of AT is that it is a source of multipotent stem cells called Adipose-derived stem cells (ADSCs, Zuk et al. 2002). Noting their regenerative potential, ADSCs are advantageous for cell-based therapies, such as autologous fat grafting (Hanson et al. 2013). Since a unique definition for ADSCs does not exist, a successful isolation of ADSCs can be determined using a typical marker panel and by the capacity of these cells to undergo multilineage differentiation (Zuk et al. 2002; Mitchell et al. 2006; Locke et al. 2009). As part of the breast tumor microenvironment (Korkaya et al. 2011), ADSCs are believed to support the growth and metastasis of cancer, while conflicting data also exist that underline an anti-tumor effect (both reviewed in Schweizer et al. (2015)). As long as the interactions between ADSCs and breast cancer cells are not fully understood, new applications in plastic surgery, wound healing, regenerative medicine, and other clinical applications (Gir et al. 2012) represent a danger of having unknown side-effects.

ADSCs exist naturally in the AT surrounding the breast tumor bed. It is traditionally treated with radiation therapy in order to destroy any remaining tumor foci as well as reduce the chance of disease recurrence (Darby et al. 2011). To achieve this goal, a whole breast irradiation is used (Albuquerque et al. 2012). More than 20 years ago, studies investigated the late side effects of radiation on breast cancer patients, who were free from recurrence, but felt decreased levels of stamina and increased fatigue (Berglund et al. 1991). An alternative strategy called partial breast irradiation, using brachytherapy, was implemented to solve this problem. During partial breast irradiation therapy, only the breast tissue around the tumor bed is targeted. In an ongoing randomized phase III study of conventional whole breast irradiation versus partial breast irradiation for women with stage 0, I, or II breast cancer (NS ABP B-39/RT0G 0413) found out that partial breast irradiation allows patients to recover from cancer-related fatigue with improved quality of life in comparison to patients with whole breast irradiation (Albuquerque et al. 2012). In contrast to the advantages of partial breast irradiation, the risk of a rise in the number of remaining tumor foci cannot be excluded. As a result of this, irradiated cells exist in the region of unirradiated cells around the tumor bed. This consequently builds the basic requirement for radiation-induced effects in non-targeted cells, called radiation-induced bystander effects (RIBEs) (Goldberg and Lehnert 2002; Iyer and Lehnert 2002). In recent years, ADSC research has gained tremendous momentum in clinical research. This is because of increasing recognition of the endogenous and high regenerative capacity of ADSCs as a benefit in applications like breast reconstruction and partial breast irradiation to decrease late side effects of radiation therapy.

Therefore, ADSCs were isolated and tested for their multilineage capacity and their expression pattern of stem cell markers. For the following investigation on general effects and RIBEs on breast cancer cell growth, tumor cells were co-cultured with ADSCs indirectly; using either a transwell system or medium transfer (MT) method.

Thus, the aim of this study was to investigate the effects of ADSCs as part of the breast tumor microenvironment on the growth and survival of tumor cells during radiation.

Experimental procedures

Isolation and culture of ADSCs

Isolation of ADSCs

The isolation of ADSCs was performed with human reduction mamoplasties from healthy female donors. This work was approved by the ethics committee at the University of Rostock, Germany (registration-number: A2017-0049). The protocol for the isolation of fat tissue has been developed and optimized from previously described work (Yang et al. 2014b; Xiong et al. 2012). Tissue samples were mechanically minced and AT was washed three times with phosphate-buffered saline (PBS, PAN-Biotech GmbH, Aidenbach, Germany) and centrifuged ($250 \times g$). After each centrifugation step, the infranatant and resultant pellet were removed. Samples were digested with 0.1% collagenase type I (100 U/mL, Gibco Life Technologies, Darmstadt, Germany) supplemented with penicillin/streptomycin (P/S, 1%; 100 X, penicillin 10,000 U/ml, streptomycin 10,000 µg/ml, Sigma-Aldrich, Steinheim, Germany) on a shaker at a low setting for 18 h at 37 °C. After complete dissociation, the tissue samples were washed with an equal volume of Dulbecco's modified Eagle medium and Kaighn's modification of Ham's F12 (DMEM-F12 media, Life Technologies) and filtered through 100 µm strainers (Greiner Bio-One, Frickenhausen, Germany). This was followed by a centrifugation step ($190 \times g$, 10 min, 37 °C) to obtain the ADSC fraction. The supernatant was discarded and the pellet was resuspended in ADSCs culture medium containing DMEM-F12 supplemented with 10% fetal bovine serum Superior (FBS, Biochrom AG, Berlin, Germany) and 1% P/S. Cells were cultured at 37 °C in the presence of 5% CO₂ for 48 h in order to enable them to adhere. Afterwards, the non-adherent fraction was removed and the remaining cells were washed twice with PBS before seeding. The medium was replaced every three days.

Cell maintenance of ADSCs

When cells reached approximately 80% confluence, the medium was discarded and cells were washed twice with PBS and trypsinized with 0.25% Trypsin/

EDTA (PAA Laboratories, Cölbe, Germany). The cell count was determined using Coulter Z2 automated cell counter (Beckmann Coulter GmbH, Krefeld, Germany). The cells were cryopreserved in ADSCs culture medium containing 10% dimethyl sulfoxide (DMSO; Merck, Darmstadt, Germany) and 20% FBS. Experiments were performed in passage three to five.

Culture of MCF-7 cells

The breast cancer cell line, MCF-7 was cultured in DMEM (Lonza BioWhittaker, Verviers, Belgium) containing 1% P/S and 10% FBS at 37 °C with 5% CO₂. Cells were passaged twice a week using 0.05% Trypsin/EDTA.

Irradiation

MCF-7 cells and ADSCs were irradiated 24 h after seeding using the Linac Siemens Oncor Expression (Healthcare Sector Siemens AG, Erlangen, Germany) at a dose rate of 3.75 Gy/min. Irradiation doses of 2, 4, 6, and 8 Gy were used, alongside a sham irradiated 0 Gy control.

Multilineage capacity of ADSCs

All multilineage differentiation protocols were taken from Zhu et al. (2013). The differentiation potential of ADSCs was investigated with differentiation kits for adipogenesis, osteogenesis, and chondrogenesis, all three from StemPro[®], Gibco Life Technologies. All kits were used according to the manufacturer's instructions. DMEM/F-12 medium supplemented with 10% heat inactivated FBS (Provitro GmbH) and 5% P/S functioned as the control medium. Differentiated cells were dyed according to the differentiation lineage: adipocytes with Oil-Red O staining, chondrocytes with Alcian Blue (both from Sigma Aldrich) and osteocytes were stained with a Von Kossa staining kit, which included a counterstaining with nuclear red (Abcam, Cambridge, UK).

Immunophenotyping of ADSCs by flow cytometry

For analysis of mesenchymal surface markers, ADSCs in passage three to five were trypsinized,

washed with staining buffer (BD Pharmingen, BD Biosciences, Heidelberg, Germany) and stained with the following antibodies against CD29-PE, CD34-PE, CD90-FITC (all BD Biosciences), CD31-PE, CD45-PE and CD106-PE (all Biolegend, London, UK) on ice and in the dark for 20 min. Fluorochrome-conjugated isotype control antibodies (BD Biosciences) were used to determine the level of non-specific binding. Samples were washed (300 g, 4 °C, 5 min) and resuspended with staining buffer. The cells were analyzed by flow cytometry directly after incubation with 7-Aminoactinomycin (7-AAD, Biolegend) for 10 min on ice and protected from light (Cytomics FC 500, Beckmann Coulter). Positive and negative events were calculated using the CXP™ software and gated for living cells (negative for 7-AAD).

Bystander effects between MCF-7 cells and ADSCs

To investigate RIBEs, cell culture inserts (Fig. 1a, Greiner Bio-One) and the medium transfer (MT, Fig. 1b) were used in the experimental design for indirect co-culture. These methods allow the detection of RIBEs, which are caused by the release of

soluble factors into the surrounding medium of ADSCs.

For transwell co-culture experiments, MCF-7 cells were seeded into 24-well plates. ADSCs were seeded into cell culture inserts within a 24-well plate containing culture medium. Immediately after irradiation with doses of 2 and 8 Gy, ADSCs were transferred into the well plates with MCF-7 cells. The cell number of MCF7 was measured in triplicate every 24 h to investigate the RIBE on cell viability.

For MT experiments, the cell culture supernatant of ADSCs, called conditioned medium (CM), was collected six hours after irradiation and centrifuged ($250 \times g$, 5 min, 20 °C). Then the CM was transferred to MCF-7 cells as a half medium exchange. This MT was used for cell proliferation and colony formation assays.

Cell proliferation assay

An equal number of both MCF-7 cells and ADSCs were seeded in quintuplicate into 96-well plates (TPP Techno Plastic Products AG, Trasadingen, Switzerland). For MT the same density of ADSCs was seeded. Radiation doses of 2 and 8 Gy were implemented. Changes in proliferation of MCF-7

Fig. 1 Experimental design for investigating the radiation induced bystander effect of adipose-derived stem cells in MCF-7 Cells: **a** Transwell co-culture system for indirect co-culture of MCF-7 cells in 24-well plates and adipose-derived stem cells (ADSCs) in ThinCerts™ were seeded 24 h before irradiation of ADSCs. Transfer of ThinCerts™ to well-plates containing MCF-7 cells was directly performed after irradiation **b** medium of unirradiated/irradiated ADSCs to MCF-7 cells was transferred 6 h after irradiation

cells were assessed by the colorimetric Cell Proliferation ELISA (Roche Diagnostics GmbH, Mannheim, Germany) and performed according to the manufacturer's instructions. Cells were incubated with bromodeoxyuridine (BrdU) for over 48 h, starting 30 min before irradiation. In order to calculate the relative BrdU Incorporation, unirradiated MCF-7 cells without MT (control) were defined as 100% BrdU incorporation.

Colony formation assay

MCF-7 cells were seeded in triplicates in T₂₅ flasks (Greiner Bio One) and treated with radiation and MT as described in Fig. 1. A full medium exchange with MCF-7 culture medium was carried out after seven days and crystal violet staining (Serva Electrophoresis GmbH, Heidelberg, Germany) was done 14 days after seeding. Stained colonies were counted to calculate the plating efficiency (PE) and survival fraction (SF) of MCF-7 cells.

Statistical analysis

All data are expressed as mean $\pm$ standard deviation (SD) or standard error of mean (SEM). To identify differences between data sets, the two tailed Student *T* test was performed. Significance was assessed at $p < 0.05$ (*#: $p < 0.05$, **: $p < 0.01$; ***: $p < 0.001$). In case of the colony formation assay, the one-tailed T-test was used, whereby significant effects were defined at $p < 0.02$ (*: $p < 0.02$, **: $p < 0.01$; ***: $p < 0.002$).

Results

Characterization of isolated ADSCs

The isolated ADSCs showed the ability to adhere to plastic and were characterized by a spindle-shaped and fibroblast-like morphology, shown in Fig. 2a. To determine the stem cell characteristics of ADSCs, the multilineage capacity and expression pattern toward certain surface markers were analyzed.

Multilineage differentiation of ADSCs

Undifferentiated ADSCs were cultured with specific differentiation medium toward adipogenic, osteogenic, and chondrogenic lineages following a minimum of 14 days of incubation. The results are illustrated in Fig. 2b. The multilineage capacity was confirmed microscopically prior to staining (a2, b2, c2) and then visualized by lineage specific staining methods (a1, b1, c1). The Oil Red O staining method revealed lipid vacuoles (bright red) in differentiated adipocytes (a1). Alcian blue confirmed the presence of glycosaminoglycan by blue staining of sulphated proteoglycans as an indicator for chondrogenesis (a2). After osteogenic differentiation, Von Kossa staining showed deposits of calcium precipitate stained grey when calcium was dispersed. It turned black when it was found in mass deposits and after an additional counterstaining with nuclear red the cell nuclei were visualized (a3). ADSCs cultured with control medium showed no induction properties for adipogenic, osteogenic, and chondrogenic differentiation (a3, b3, and c3).

Expression pattern of ADSCs

The expression patterns of ADSCs were analyzed by flow cytometry for specific surface markers at passage three to five of three different donor materials. As expected, CD29 and CD90 were expressed in nearly every way whereas CD31, CD34, CD45 and CD106 were not (Fig. 2c). This expression profile did not change between passage three to passage five (Figure in supplement, S1).

Transwell co-culture of MCF-7 Cells and ADSCs

Growth Curve

To analyze the RIBEs of irradiated ADSCs on their neighboring unirradiated MCF-7 cells, a transwell co-culture system was used. The number of co-cultured MCF-7 cells was measured daily and used to calculate the population doubling time during the period of logarithmic MCF-7 growth from day two to six (Fig. 3). While sham and medium dose (2 Gy) irradiated ADSCs accelerated the growth of co-cultured MCF-7 cells, high dose (8 Gy) irradiated ADSCs led to decreased MCF-7 cell numbers on the

Fig. 2 Characterization of isolated human adipose derived stem cells. **a** Morphology of human adipose-derived stem cells (ADSCs) isolated from breast tissue 3 four days after seeding at passage 3 (scale bar: 500 μ m) **b** Trilineage differentiation capacity of ADSCs. **a** Adipogenic differentiation: Differentiated adipocytes (1) were stained with Oil red O (2, lipid droplets are shown as bright red globules) and undifferentiated ADSCs were stained as a control (3). **b** Chondrogenic differentiation: Differentiated chondrocytes (2) and undifferentiated ADSCs as control (3) stained with Alcian blue, blue

regions showing sulphated proteoglycans—an indicator for chondrogenesis **c** Osteogenic differentiation: Differentiated osteocytes (1, 2) and undifferentiated ADSCs as control (3) stained with Von Kossa Kit (2 and 3), calcium precipitate regions were distinguishable by a dark brown color. **c** Expression pattern of ADSCs from three different donors were tested towards the surface cell markers CD29, CD31, CD34, CD45, CD90 and CD106 by flow cytometry. Results are illustrated as mean $\pm$ standard error of the mean (SEM, $n = 3$). (Color figure online)

days four, five and seven (Fig. 3a). Those effects are reflected in the PDT of co-cultured MCF-7 cells that decrease with increasing radiation dose of ADSCs from 32.4 h (0 Gy) to 35.1 h (8 Gy).

Medium transfer (MT) experiments from ADSCs to MCF-7 cells

To investigate the short-term effects of radiation on ADSCs, the MT procedure was carried out six hours after irradiation as a half medium exchange of MCF-7 cells with ADSCs CM. The CM contains any factor released from the ADSCs, which in turn affect MCF-7 cells. The different experimental approaches have been illustrated previously (Fig. 1).

Clonogenic survival

In order to examine the long-term effects following radiation exposure and treatment with ADSCs CM, the clonogenic survival of MCF-7 cells was measured 14 days after different treatment combinations (Fig. 1). The resulting survival fractions (SF) of MCF-7 cells were calculated by the relative number of surviving colonies. These were normalized after measuring MCF-7 cells that were not treated with ADSCs CM (control, Fig. 4).

In general, the number of MCF-7 colonies decreased upon increasing doses of radiation (Fig. 4b). A typical shoulder curve was observed within the low dose ranges up to 4 Gy. It progressed into an exponential curve after IR with higher doses (Fig. 4b). The treatment with CM from irradiated or unirradiated ADSCs did not change those

Fig. 3 Growth characteristics of MCF-7 cells in co-culture with differently irradiated adipose derived stem cells. Triplicates of 5×10^3 MCF-7 cells were seeded in 24-well plates and 1×10^3 adipose-derived stem cells (ADSCs) in cell culture inserts; 1 day after seeding ADSCs were irradiated with different doses (0 Gy, 2 Gy, 8 Gy) and transferred directly to MCF-7 cells (on day 1). Daily cell count of MCF-7 cells for over 7 days were performed and the results of the co-cultures of MCF-7 cells with ADSCs from 5 different donors are illustrated as mean $\pm$ standard error of the mean (SEM, $n = 5$); $^*/\#/+$ $p < 0.05$ and $##$ $p < 0.001$ significant differences to * control, $\#$ MCF-7 cells in co-culture with 2 Gy irradiated ADSCs and $+$ MCF-7 cells in co-culture with 8 Gy irradiated ADSCs. MCF-7 cell numbers were used to calculate the population doubling time (PDT) of logarithmic growth from second day to sixth day, illustrated as mean $\pm$ SEM

characteristics. In the second approach, unirradiated MCF-7 cells were treated with CM from irradiated ADSCs (Fig. 4a). This led to an average decrease in MCF-7 survival fraction of around 14%, independent of radiation dose (2–8 Gy).

Proliferation analysis (BrdU)

The short-term effects of radiation and the influence of ADSCs CM on MCF-7 proliferation involved the BrdU incorporation assay, which determines cell

divisions. These cell divisions were documented for 48 h, starting at the time-point of radiation (Fig. 5).

The MT from unirradiated ADSCs to MCF-7 cells, six hours after irradiation, caused small fluctuations, but no significant changes in the proliferation of MCF-7 cells.

Discussion

Based on our findings, we postulate that interactions of ADSCs and breast cancer cells are influenced by radiation therapy.

We view this as an important basis for new trials especially since the use of ADSCs containing lipografts for breast reconstruction are becoming increasingly more conventional in clinical practice. In spite of its advantages for reconstructive purposes, numerous investigations describe the promoting effects of ADSCs on residing cancer cells (Eterno et al. 2014; Yang et al. 2014a; Kuhbier et al. 2014; Trivanović et al. 2016). But so far the relevant mechanisms remain poorly understood. Bystander effects can occur from communication between cells through direct cell–cell contacts or via transmissible molecules secreted into the medium of the targeted cells. One possible explanation of promoting effects of ADSCs on residing cancer cells seems to be attributed to the wide range of cytokines, which were shown to be released by ADSCs (Rehman et al. 2004; Melief et al. 2013; Kokai et al. 2014). Therefore altered cytokine production of ADSCs after co-culturing with breast cancer cells was detected (Eterno et al. 2014). Likewise, with inflammatory cytokines primed ADSCs stimulate the migration and invasion capability of MCF-7 breast cancer cells via TGF- β 1 (Trivanović et al. 2016).

On the basis of these findings, we decided to use indirect co-culture systems to analyze the interactions between ADSCs and breast cancer cells via soluble factors. While transwell co-culture systems enable a bidirectional exchange, the medium transfer (MT) method only allows a unidirectional interaction. Following our investigation, these differences in the influence of ADSCs on breast cancer cells between two co-culture systems were not observed. This indicated that breast cancer cells in short-term co-culture do not change the radiation-induced negative effects of ADSCs. In contrast, Trivanovic et al.

Fig. 4 Colony forming efficiency assay of irradiated and unirradiated MCF-7 cells after medium transfer from adipose-derived stem cells. Triplicates of MCF-7 cells (5×10^3) and adipose-derived stem cells (ADSCs, 13×10^4) were seeded 24 h before irradiation in cell culture flasks. Six hours after irradiation a medium transfer with ADSCs conditioned medium (CM) for unirradiated (a) or irradiated (b) MCF-7

cells was performed, followed by a full medium exchange with culture medium on day 8 and crystal violet staining on day 15. MCF-7 cells survival fractions (SF) of the different experimental approaches were normalized to the control; results are illustrated as mean $\pm$ standard deviation (SD, $n = 3$), *: $p < 0.02$, **: $p < 0.01$; ***: $p < 0.002$

(2014) reported a proliferation stimulation on MCF-7 cells in direct mixed co-culture and transwell systems, but an antiproliferative effect using a conditioned medium. Therefore, Trivanovic and co-authors concluded that supporting and inhibiting effects of ADSCs on tumor cells seem to be dependent on the co-culture system whereas RIBEs are not. In contrast to their results we could not detect any antiproliferative effect on breast cancer cells using conditioned medium. This discrepancy may originate from different experimental designs of both studies. Whereas in our study the breast cancer cells were conditioned with medium of irradiated ADSCs, Trivanovic and co-worker treated tumor cells with

medium of untreated ADSCs and irradiated after medium transfer.

We demonstrated a supporting effect of unirradiated or with low doses treated ADSCs on the growth of breast cancer cells whereas higher radiation doses inhibited the tumor cell growth. On the other hand irradiated ADSCs reduced the survival of untreated tumor cells, independent of radiation dose. Yang et al. (2014a) used a comparable experimental design and postulated that ADSCs have a radioprotective effect on breast cancer cells. In contrast to the present study, MCF-7 cells were treated with supernatant of unirradiated ADSCs before irradiation. With regard to our results, it is conceivable that a second MT after

Fig. 5 BrdU-Incorporation in MCF-7 cells after irradiation and treatment with conditioned medium from adipose-derived stem cells. Conditioned medium from irradiated adipose-derived stem cells (ADSCs, 0 Gy, 2 and 8 Gy) of four different

donors to MCF-7 cells six hours after irradiation; BrdU-incorporation was measured 48 h after irradiation; results are illustrated as mean $\pm$ standard deviation (SD, $n = 4$)

irradiation of ADSCs could neutralize this radioprotective effect on breast cancer cells.

There is also an inconsistency in publications that describe the promoting (Yang et al. 2014a; Kuhbier et al. 2014; Trivanović et al. 2016) and suppressing effects of ADSCs on breast cancer cells (Ryu et al. 2014; Trivanovic et al. 2014, 2016; Lee et al. 2015). This could be caused by the use of different radiation doses like in our study concerning the tumor cell growth. As shown, cell proliferation of MCF-7 cells is not affected and could not be the reason for the effects of ADSCs on tumor cell growth. It also could be due to the wide range of ADSCs isolation, cultivation, and co-culture methods (Klopp et al. 2011). The successful isolation of ADSCs can be determined using a distinctive cell surface marker panel and by the capacity of these cells to undergo multi lineage differentiation (Zuk et al. 2002; Mitchell et al. 2006; Locke et al. 2009). Differentiation potential in our study was proven by inducing various differentiation lineages. According to Zhu et al. (2013), a cell population that can be induced into these lineages are indicative of stem cells, as this is solely a stem cell characteristic. The second criterion for the successful isolation of ADSCs in the present study was achieved by the detection of CD90 and CD29 and lack of CD31, CD34, CD45 and CD106 as specific surface markers. Another source for discrepancy of effects in the literature could be caused by different sources of adipose tissue (AT),

like brown, mammary and white AT with different particularities (Kolaparthi et al. 2015). In our study, ADSCs isolated from healthy breast tissue from human reduction mammoplasties of female donors, were used as a model for endogenous stem cells in the vicinity of residing breast cancer cells. Because functional variations among ADSCs from different origins have already been described (Hanson et al. 2013; Chen et al. 2015), we also investigated the proliferation and colony formation ability of MCF-7 cells co-cultured with commercially available human derived ADSCs (Invitrogen™, Fisher Scientific) sourced from the abdomen. No qualitative differences were recognized (data not shown). Therefore, our results are also transferable to issues of fat grafting, where abdominal AT is used.

Conclusion

In conclusion, the present study demonstrates clearly that irradiation influences the interaction of ADSCs and breast cancer cells. Unirradiated or with lower doses treated ADSCs improved the growth of tumor cells, whereas using higher radiation doses resulted in a significant inhibition of tumor cell growth. Furthermore medium from irradiated ADSCs reduced clonogenic survival of untreated breast cancer cells, but not in irradiated tumor cells. This finding highlights the importance of further studies to

exclude potential risks of ADSCs in regenerative applications and radiotherapy. To work out the optimal conditions and those ADSCs inhibit cancer cells could be the key for the safe and effective use of ADSCs in regenerative medicine.

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Compliance with ethical standards

Conflict of interests The authors have no conflict of interest to declare.

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