RESEARCH ARTICLE

Effects of umbilical cord- and adipose-derived stem cell secretomes on ALDH1A3 expression and autocrine TGF- β 1 signaling in human breast cancer stem cells [version 1; peer review: 1 approved with reservations, 1 not approved]

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Abstract

Background: Nowadays, umbilical cord- and adipose-derived stem cells (UCSCs and ASCs) are the most common sources of mesenchymal stem cells (MSCs). As part of the tumor microenvironment, MSCs are known to communicate with cancer cells via their secretomes. Increased activity of aldehyde dehydrogenase-1 (ALDH1) has been widely used as a common intrinsic stemness marker in normal and cancer stem cells. Our study aimed to elaborate on the effect of UCSC and ASC secretomes on the expression of ALDH1A3, as one of the important variants of ALDH1, TGF- β 1 and TGF- β receptor type I (T β RI) in human breast cancer stem cells (BCSCs).

Methods: UCSCs and ASCs were cultured in serum-free α -MEM media under standard conditions for 24 hours. The conditioned medium (CM) containing secretomes of UCSCs and ASCs were collected and added 50% (v/v) to the cultured of human BCSCs for 72 hours. The mRNA expressions of ALDH1A3, TGF- β 1, and T β RI were determined using quantitative Reverse Transcriptase Polymerase Chain Reaction (q-RT-PCR).

Results: We found that CM of UCSCs significantly increased the ALDH1A3 expression of BCSCs in parallel with the increase of TGF- β 1 and T β RI expressions. Conversely, CM of ASCs had no significant effect on the ALDH1A3 expression, but significantly decreased TGF- β 1 and T β RI expressions of BCSCs. These results contradict our published data on ALDH1A1, which is another important variant of ALDH1, as well as data of the pluripotency markers OCT4 and SOX2 expressions.

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Approval Status ? X

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1. Albert D. Donnenberg, University of Pittsburgh Cancer Institute, Pittsburgh, USA University of Pittsburgh, Pittsburgh, USA University of Pittsburgh, Pittsburgh, USA 2. Paola Marcato, Dalhousie University, Halifax, Canada Any reports and responses or comments on the article can be found at the end of the article.		

Conclusions: UCSC and ASC secretomes have different regulation on ALDH1A3 expression in human BCSCs, which may be related to the autocrine TGF- β 1 signaling in modulating cell proliferation and stemness of BCSCs. Further studies are required to evaluate factors involved in the differential effects of UCSC and ASC secretomes that regulate ALDH1A3 expression in relation to autocrine TGF- β 1 signaling and aggressiveness of human BCSCs.

Keywords

UCSCs, ASCs, BCSCs, ALDH1A3, ALDH1A1, TGF- β 1, T β RI , pluripotency

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Introduction

Mesenchymal stem cells (MSCs) are non-hematopoietic and adherent cells characterized by high CD90, CD105 and CD73, but lack of CD14, CD34 and CD45 expression. When treated with certain differentiation-stimulating factors, these cells will differentiate into adipocytes, chondrocytes, as well as osteocytes. MSCs show clinical feasibility, as studies on multiple animal models that yielded therapeutic efficacy gave rise to a series of clinical trials in a wide range of major diseases¹. Umbilical cord and adipose tissue are two of the most common sources of MSCs, due to the fact that they can be non-invasively obtained and have minimal risk in terms of immunological and ethical issues. Apart from sharing the common properties of MSCs, umbilical cord-derived stem cells (UCSCs) are reported to have higher proliferation capability than adipose-derived stem cells (ASCs), whereas ASCs are able to differentiate to adipose tissue better than UCSCs². Very recently, we have demonstrated that both ASCs and UCSCs expressed lower ALDH1A1 and OCT-4 than breast cancer stem cells (BCSCs), indicating that both types of MSCs have lower pluripotency compared to BCSCs (Wanandi SI, Purnamawati, Tamara A, Putri KT, Simadibrata D, *in press*).

Nowadays, MSCs are widely used for regenerative therapies due to their multipotent differentiation capacity. On top of that, MSCs are capable to secrete various paracrine signals for tissue regeneration and revascularization, such as chemoattractive, immunomodulatory, angiogenic, anti-apoptotic, and pro-survival factors. Nevertheless, MSC secretomes have also been reported to promote cancer progression and metastasis³. Accumulating evidence of cell-cell and paracrine interactions between MSC and cancer cells has indicated that MSCs can either induce or inhibit tumor progression³⁻⁹. Secretomes contained in conditioned medium (CM) of MSCs consist of various biologically active factors that have the same effectiveness as MSCs themselves¹⁰. In addition, secretomes of MSCs are considered safer to use since they do not contain cellular elements,¹¹ making them free from possible mutations and transformation into cancer-associated fibroblast in a cancer microenvironment¹².

Normal and cancer stem cells share similar properties, such as self-renewal capacity and differentiation potential into multiple cell types. Activity of aldehyde dehydrogenase (ALDH) superfamily have been widely used as a marker of viable normal stem cells, as well as cancer stem cells¹³. In breast cancer, the existence of ALDH+ BCSCs often correlates with poor prognosis, progression, chemoradiation resistance, and metastasis. Chemotherapy resistance is due to the role of ALDH as a detoxifying enzyme, which mediates detoxification of toxic aldehyde intermediates that are produced in certain chemotherapeutic agents-treated cancer cells, while radioresistance occurs through direct removal of oxygen radicals and indirect production of antioxidant compound nicotinamide adenine dinucleotide (phosphate)¹⁴.

ALDH1A1 and ALDH1A3, two of ALDH1 superfamily isozymes, have been known to play important roles in the modulation of the retinoic acid signaling pathway, which can either support or

suppress cancer growth¹⁴⁻¹⁶. In addition, they also serve as markers of stemness in CSCs^{13,17}. The expression of ALDH1A1 was associated with advanced, triple-negative, and poor prognosis breast cancer after neoadjuvant chemotherapy^{18,19}. ALDH1A1 expression was also found to be predictive of tumor responsiveness to cyclophosphamide treatment^{20,21}. Meanwhile, the isoform of ALDH1A3 has been suggested to promote breast cancer progression via retinoic acid signaling²².

TGF- β family members play a major regulatory role in various biological and physiological functions²³. TGF- β physiologically exerts anticancer activities in normal and benign cells by prohibiting cell proliferation and by creating cell microenvironments that inhibit cell motility, invasion, and metastasis. However, along with the development and progression of tumors, various mutations or deletions occur in genes that encode various TGF- β signaling components. These lead to the loss of protective and cytostatic effects of TGF- β , which in turn alters TGF- β signaling to promote cancer progression, invasion, and tumor metastasis^{23,24}. Recently, the mechanism of the TGF- β paradox may have been explained through Erk activation, which will auto-induce TGF- β in the microenvironment²⁵. Auto-induction of TGF- β in benign or early stage cancer cells will create negative feedback of TGF- β signaling leading to growth arrest, whereas progression and metastasis in malignant cancer cells will be induced via a positive feedback loop²⁵.

Until now, the interaction between MSC secretomes and BCSCs has not been fully understood. Our recent study has indicated that BCSCs treated with secretomes of ASCs expressed ALDH1A1 significantly higher compared to those treated with UCSCs, in accordance to OCT-4 and SOX2 expressions²⁶. Those results showed that secretomes of ASCs contribute to pluripotency and viability of BCSCs more than those of UCSCs. To gain a better understanding of the effects of MSC secretomes on BCSCs, we conducted the present study on ALDH1A3 expression in association with TGF- β 1 signaling pathways in BCSCs.

Methods

Ethics and specimens

According to the Declaration of Helsinki 1964, this study has been approved by the Health Research Ethics Committee Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital (No. 205/UN2.F1/ETIK/2016).

MSC specimens consisting of three ASCs and three UCSCs samples were obtained from HayandraLab and Stem Cell Medical Technology Integrated Service Unit, Cipto Mangunkusumo Central Hospital Faculty of Medicine Universitas Indonesia, Jakarta Indonesia, and have been characterized by the expression of stromal cell markers, i.e. CD73, CD90 positive and CD34 negative, as well as multidifferentiation capabilities to osteogenic, chondrogenic and adipocyte lineages as reported in our previous study^{26,27}.

BCSCs (ALDH+) were obtained from Cell Culture Laboratory for Cancer Stem Cells, Department Biochemistry and Molecular Biology Faculty of Medicine Universitas Indonesia, Jakarta

Indonesia. The BCSC specimen has been isolated by ALDEFUOR™ assay for subsequent downstream assessment (Fluorescence Activated Cell Sorting in Tsukuba University, 2015).

Cell cultures

MSCs were grown in Minimum Essential Medium Alpha (α -MEM) supplemented with 10% FBS, while BCSCs were grown in non-serum DMEM/F12 medium. Both cell cultures were supplemented with 1% penicillin-streptomycin and 1% amphotericin. Cells were incubated under standard conditions (5% CO₂ and 37°C). The media were replaced every 3 days and cells were subcultured when confluence was obtained.

Preparation of MSC-conditioned medium (MSC-CM)

Early-passage human MSCs (P3-P5) were grown to 70–80% confluence in α -MEM medium with 10% FBS. Culture medium was removed and the cells were washed three times by PBS 1x to remove any residual serum. The cells were then grown in non-serum α -MEM medium under standard conditions for 24 hours. MSC-CM was centrifuged at 200xg for 10 min to remove cell debris and filtered using 0.22 μ m filters. Subsequently, CM was stored at -20°C and used within 3 days. To prepare the CM for each experiment, CM was diluted with DMEM/F12 to obtain a 50% (v/v) concentration.

Incubation of BCSCs (ALDH+) with MSC-CM and viability assay

BCSCs (ALDH+) were harvested and counted using an automatic cell counter (Luna®). About 5 x 10⁵ cells with more than 90% viability were grown in non-serum DMEM/F12 medium. After 24 hours, the BCSC medium was replaced with 50% (v/v) MSCs-CM. After 72 hours of incubation, cells were harvested and counted using Trypan blue exclusion dye assay. Cells that were grown in 50% (v/v) non-serum α -MEM medium were used as a control. The experiment was performed in triplicate.

RNA isolation and qRT-PCR assay

Isolation of total RNA was performed according to the manufacturer's instruction (TriPure Isolation Reagent®, Roche). The RNA concentration was measured using a MicroDrop

spectrophotometer (Thermo Scientific Skanlt Software for Varioskan™ Flash Multimode Reader). Quantitative reverse transcriptase PCR was performed using SYBR Green and reverse transcriptase enzyme (One-Step qRT-PCR Kit KAPA™SYBR@FAST). The cycling conditions were 5 minutes at 42°C for cDNA synthesis, 5 minutes at 95°C for inactivation of reverse transcriptase enzyme, then 40 cycles consisting of 30 seconds at 95°C for double stranded denaturation and 20 seconds at annealing gene temperature optimized for annealing stage, 20 seconds at 72°C for elongation stage. 18S rRNA was used as an internal control. The normalized fold expression was obtained using the 2^{- $\Delta\Delta$ CT} (Livak) method. Primers used for qRT-PCR were obtained from IDT® (Table 1).

Statistical analysis

All relative gene expression data were analyzed using unpaired Student's t-test, SPSS 20 and presented as mean $\pm$ standard error.

Results

Morphologies of MSCs and BCSCs

BCSCs showed sphere formation within 2–3 days after plating (Figure 1a). UCSCs and ASCs used in this study adhered within hours after plating and displayed spindle-fibroblast-like morphology (Figure 1b–c). Both MSCs gradually fused into a single layer after cell confluence has been reached. Multidifferentiation capability of ASCs and UCSCs have been verified in our previous study^{26,27}.

Dataset 1. Raw unedited images for Figure 1A, 1B, and 1C

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Expression of ALDH1A3 gene in BCSCs treated with CM of UCSCs and ASCs (Figure 2)

Treatment of UCSC-CM to BCSCs could significantly increase the relative expression of ALDH1A3 gene (1.79 times, p=0.001) compared with the control cells grown in 50% (v/v) non-serum α -MEM medium. In contrast, ASC-CM had no significant effect on ALDH1A3 gene expression in BCSCs (1.18 times, p=0.316) compared with the control cells. Nevertheless, the

Table 1. Primer sequences used in the qRT-PCR.

Gene	Primer Forward	Primer Reverse	Amplicon Length (bp)
ALDH1A3	5'-CGA CCT GGA GGG CTG TAT TA-3'	5'-TGG TGA AGC ACA CGA CGT T-3'	104
TGF- β 1	5'-GCC TTT CCT GCT TCT CAT GG-3'	5'-CTC CGT GGA GCT GAA GCA ATA-3'	105
T β RI	5'- ACT TCC AAC TAC TGG CCC TTT-3'	5'-AGA TGC AGA CGA AGC ACA CT-3'	100
18S rRNA	5'- AAA CGG CTA CCA CAT CCA AG-3'	5'-CCT CCA ATG GAT CCT CGT TA-3'	155

Figure 1. Morphologies of BCSCs (a), UCSCs (b), and ASCs (c). About 1×10^5 cells were plated in each well of a 12-well plate and were grown under standard conditions as described under Materials and Methods. After 2–3 day incubation, cell morphology was observed under inverted microscope at 100x magnification. BCSCs, breast cancer stem cells; UCSCs, umbilical cord-derived stem cells; ASCs, adipose-derived stem cells; CM, conditioned medium.

Figure 2. Relative expression of ALDH1A3 mRNA in BCSCs. Human BCSCs were treated with 50% (v/v) UCSC-CM and ASC-CM, respectively. As a control, BCSCs were treated in 50% (v/v) non-serum α -MEM medium. After 72-hour incubation, total RNA was isolated and quantitative reverse transcriptase PCR was performed to determine ALDH1A3 mRNA expression levels in human BCSCs. The Cq obtained was normalized to 18S rRNA and control cells. Data is presented as mean $\pm$ SE. Significance differences are considered at * $p < 0.05$; ** $p < 0.01$ (Student's t-test). BCSCs, breast cancer stem cells; UCSCs, umbilical cord-derived stem cells; ASCs, adipose-derived stem cells; CM, conditioned medium.

effect of ASC- was significantly lower than that of UCSC-CM ($p=0.024$). These results indicate that ALDH1A3 was distinctly expressed in BCSCs treated with UCSC- and ASC-CM.

Dataset 2. Data for Figure 2 (ALDH1A3 Cq value)

<http://dx.doi.org/10.5256/f1000research.13609.d194563>

ALDH1A3 Cq was used to calculate ALDH1A3 mRNA expression levels using Livak formula as demonstrated in Figure 2 (Control: 50% (v/v) a-MEM-treated cells; UCSC-CM: conditioned medium of umbilical cord-derived stem cells; ASC-CM: conditioned medium of adipose-derived stem cells).

Expression of TGF- β 1 and T β RI genes in BCSCs treated with CM of UCSCs and ASCs (Figure 3)

In attempt to analyze the effect of MSC-CM on TGF- β signaling, we determined the relative mRNA expressions of TGF- β 1 and its receptor, T β RI. UCSC-CM significantly increases the relative expression of TGF- β 1 (1.72 times, $p=0.003$) and T β RI (1.54 times, $p=0.000$). In contrast to this data, ASCs-CM significantly decreases the expression of TGF- β 1 (0.64, $p=0.003$) and T β RI (0.76, $p=0.014$) in BCSCs compared with controls. Additionally, UCSC- and ASC-CM showed differential effects on either TGF- β 1 ($p=0.000$) or T β RI ($p=0.000$) expression of BCSCs, suggesting that UCSC and ASC secretomes may be different in content and levels of growth factors, thereby influencing differential regulation of TGF- β 1 and T β RI expressions.

Dataset 3. Data for Figure 3A (TGF-b1 Cq value)

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TGF-b1 Cq was used to calculate TGF-b1 mRNA expression levels using Livak formula as demonstrated in Figure 3A (Control: 50% (v/v) a-MEM-treated cells; UCSC-CM: conditioned medium of umbilical cord-derived stem cells; ASC-CM: conditioned medium of adipose-derived stem cells).

Dataset 4. Data for Figure 3B (TbRI Cq value)

<http://dx.doi.org/10.5256/f1000research.13609.d194565>

TbRI Cq was used to calculate TbRI mRNA expression levels using Livak formula as demonstrated in Figure 3B (Control: 50% (v/v) a-MEM-treated cells; UCSC-CM: conditioned medium of umbilical cord-derived stem cells; ASC-CM: conditioned medium of adipose-derived stem cells).

Dataset 5. Data for Figures 2, 3A, and 3B (18S rRNA Cq value)

<http://dx.doi.org/10.5256/f1000research.13609.d194566>

18S rRNA Cq was used to calculate ALDH1A3, TGF-b1, and TbRI mRNA expression levels using Livak formula (Control: 50% (v/v) a-MEM-treated cells; UCSC-CM: conditioned medium of umbilical cord-derived stem cells; ASC-CM: conditioned medium of adipose-derived stem cells).

Figure 3. Relative expression of TGF- β 1 (A) and T β RI (B) mRNA in BCSCs. Human BCSCs were treated with 50% (v/v) UCSC-CM and ASC-CM, respectively. As a control, BCSCs were treated in 50% (v/v) non-serum α -MEM medium. After 72-hour incubation, total RNA was isolated and quantitative reverse transcriptase PCR was performed to determine TGF- β 1 and T β RI mRNA expression levels in human BCSCs. The Cq obtained was normalized to 18S rRNA and control cells. Data is presented as mean $\pm$ SE. Significance differences are considered at * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Student's t-test). BCSCs, breast cancer stem cells; UCSCs, umbilical cord-derived stem cells; ASCs, adipose-derived stem cells; CM, conditioned medium.

Discussion

Recently, the utilization of secretomes contained within MSC-CM has begun to be performed on various rejuvenation therapies. However, the possible consequences that may arise due to the interaction between biologically active factors (secretomes) and cancer cells has not yet been elucidated. As with MSCs, their secretomes have also been reported to either suppress or promote growth and development of cancer³. These contradictory effects may be due to different composition of secretomes, with one of the causes being the fact that they are obtained from different MSC sources^{4,28}.

In this study, we found that secretomes within UCSC-CM have a higher ability to promote ALDH1A3 gene expression in BCSCs, indicating the higher paracrine signaling activity of UCSC secretomes when compared to those of ASCs (Figure 2). This result is in contrast with the effect of MSC secretomes on ALDH1A1 expression of BCSCs demonstrated in our previous study, which revealed that ALDH1A1 mRNA expression was significantly reduced by UCSC-CM and increased by ASC-CM²⁶. In that study, we also found that ALDH1A1 expression is in line with the expression of OCT-4 and SOX2. Therefore, we suggest that unlike ALDH1A1, ALDH1A3 does not prominently contribute to the pluripotency of BCSCs. This has been verified by *in silico* analysis that showed that there is no direct interaction between ALDH1A3 and pluripotency markers, OCT4, SOX2, NANOG, and KLF4 (in press; Wanandi SI, Purnamawati, Tamara A, Putri KT, Simadibrata D). That study has also indicated that ALDH1A1 expression levels in MSCs were more similar to OCT4 rather than to ALDH1A3 levels, suggesting the role of ALDH1A1 on pluripotency.

In the present study, we highlighted that the effects of UCSC and ASC secretomes on ALDH1A3 were consistent with the expressions of TGF- β 1 and its receptor, T β RI, in BCSCs (Figure 3). In spite of that, the effects of UCSC on ALDH1A3, TGF- β 1, and T β RI expressions were opposite to those of ASC secretomes, in which UCSC up-regulated, while ASC secretomes down-regulated those gene expressions. Previous studies have reported that different MSC sources have different growth factor contents and levels^{4,28}. Very recently, we have also demonstrated that UCSCs and ASCs expressed different levels of either ALDH1A1 or ALDH1A3 (in press; <Wanandi SI, Purnamawati, Tamara A, Putri KT, Simadibrata D>). These two isozymes of ALDH1 have newly been confirmed to have differential functional roles in facilitating aggressiveness of human breast cancer cells²⁹. ALDH1A1 suppresses proliferation, metastatic properties and therapy resistance of breast cancer cells, whereas ALDH1A3 has predominant effect on ALDH activity. These results supported our previous study that presented the increase of viability and pluripotency in ASC secretomes-treated BCSCs in line with the increase of ALDH1A1²⁶.

Moreover, the current study also underlines the effect of UCSC and ASC secretomes on TGF- β 1 autocrine signaling in BCSCs, as revealed by TGF- β 1 and its receptor, T β RI expressions (Figures 3A and B). We suggest that secretomes of UCSCs and ASCs presumably contained growth factors

including TGF- β 1 that could auto-induce TGF- β 1 signaling in BCSCs. Due to TGF- β 1 paradox, tumor proliferation could be stimulated or inhibited either via differential ERK depending on relative level of TGF- β 1 present in tumor microenvironment²⁵. A plausible explanation of TGF- β 1 autocrine signaling induction in our BCSCs is as a cellular homeostasis against reduced cell viability and pluripotency due to enhanced ALDH1A3 and diminished ALDH1A1 expression after supplementation with UCSC secretomes^{26,28}.

In conclusion, the differential effects of UCSC and ASC secretomes on ALDH1A3 expression in human BCSCs may be related to autocrine TGF- β 1 signaling, as opposed to ALDH1A1 which regulates BCSC viability and pluripotency. In depth studies are further required to elaborate signaling factors within UCSC and ASC secretomes that specifically regulate ALDH1A1 and ALDH1A3 expressions in relation to TGF- β 1 autocrine signaling and its impact on the aggressiveness of BCSCs.

Data availability

Dataset 1: Raw unedited images for Figure 1A, 1B, and 1C. DOI, [10.5256/f1000research.13609.d194562](https://doi.org/10.5256/f1000research.13609.d194562)³⁰

Dataset 2: Data for Figure 2 (ALDH1A3 Cq value). ALDH1A3 Cq was used to calculate ALDH1A3 mRNA expression levels using Livak formula as demonstrated in Figure 2 (Control: 50% (v/v) α -MEM-treated cells; UCSC-CM: conditioned medium of umbilical cord-derived stem cells; ASC-CM: conditioned medium of adipose-derived stem cells). DOI, [10.5256/f1000research.13609.d194563](https://doi.org/10.5256/f1000research.13609.d194563)³¹

Dataset 3: Data for Figure 3A (TGF- β 1 Cq value). TGF- β 1 Cq was used to calculate TGF- β 1 mRNA expression levels using Livak formula as demonstrated in Figure 3A (Control: 50% (v/v) α -MEM-treated cells; UCSC-CM: conditioned medium of umbilical cord-derived stem cells; ASC-CM: conditioned medium of adipose-derived stem cells). DOI, [10.5256/f1000research.13609.d194564](https://doi.org/10.5256/f1000research.13609.d194564)³²

Dataset 4: Data for Figure 3B (T β RI Cq value). T β RICq was used to calculate T β RI mRNA expression levels using Livak formula as demonstrated in Figure 3B (Control: 50% (v/v) α -MEM-treated cells; UCSC-CM: conditioned medium of umbilical cord-derived stem cells; ASC-CM: conditioned medium of adipose-derived stem cells). DOI, [10.5256/f1000research.13609.d194565](https://doi.org/10.5256/f1000research.13609.d194565)³³

Dataset 5: Data for Figure 2, Figure 3A, and Figure 3B (18S rRNA Cq value). 18S rRNA Cq was used to calculate ALDH1A3, TGF- β 1, and T β RI mRNA expression levels using Livak formula (Control: 50% (v/v) α -MEM-treated cells; UCSC-CM: conditioned medium of umbilical cord-derived stem cells; ASC-CM: conditioned medium of adipose-derived stem cells). DOI, [10.5256/f1000research.13609.d194566](https://doi.org/10.5256/f1000research.13609.d194566)³⁴

Competing interests

No competing interests were disclosed.

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[Data Source](#)

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Paola Marcato

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This results presented by Purnamawati et al., are potentially interesting; however, since the key findings of the paper hinges on the fact that breast cancer stem cells were used in the assays it is difficult to interpret the findings (i.e. no details of the source of cells or proof they are cancer stem cells was provided).

Major concerns.

1. There is no details of the source of the cells, nor was the Aldefluor sorting of the cells shown.
2. Assay were not performed to confirm that cancer stem cells had been isolated (i.e. are these cells more tumorigenic than non-cancer stem cell counterparts?
3. Expression of ALDH1A3 should be compared in in the breast cancer stem cell versus non-cancer stem cell sorted populations to confirm that the Aldefluor+ cells have higher levels of ALHD1A3 than the Aldefluor- cells.
4. The culture conditions used to form mammospheres were not appropriate (should seed much lower number of cells in ultra-low adherent plates so that no cells are adherent.) Standard media for mammosphere assays in Mammoscult media from Stemcell Technologies. Hence it is unclear if these are mammospheres.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

No

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 29 May 2018

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Albert D. Donnenberg

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This study investigated the effects of media conditioned by umbilical cord (UC)-derived and adipose-derived stromal cells on "breast cancer stem cells." The measured outcomes were aldehyde dehydrogenase 1A3 (ALDH1A3), TGF- β 1 and T β R1 messenger RNA. The methodology and data analysis are straight-forward and sound. The major finding was that culture in the presence of umbilical cord-derived stromal cell conditioned medium resulted in higher expression of ALDH1A3, TGF- β 1, and T β R1 mRNA, compared to control medium. In contrast, adipose stromal cell conditioned medium resulted in slightly lower expression of TGF- β 1, and T β R1 mRNA, but this was a modest effect. The authors conclude that different secretomes of UC- and adipose-derived stromal cells differentially regulate ALDH1A3 expression in breast cancer "stem cells", a point that is well-supported by their results. They further conclude that differential regulation of ALDH1A3 may be related to autocrine TGF- β 1 signalling in modulating cell proliferation and stemness of breast cancer stem cells. This is speculative and based on a partial correlation of expression levels of the three measured mRNAs. If anything, the data speak against a causal relationship, as adipose-conditioned medium failed to down-regulate ALDH1A3 message, but modestly downregulated TGF- β 1, and T β R1 mRNA. To their credit, the authors point out that the present results are inconsistent with their previous published data on ALDH1A1, OCT4 and SOX2 expression.

These results speak to the difficulty of modeling the interactions of stromal cells and cancer cells. Using freshly isolated human breast cancer cells, flow-sorted on the basis light scatter and expression of CD90, we found that co-injection of adipose stromal cells in a xenograft model increased tumorigenicity of active, but not resting tumor cells. In vitro, coculture of unpassaged

breast cancer cells with adipose stromal cells greatly increased their proliferation¹. The take-home is that the state of the cancer cells matters as much as the signals provided by the stromal cells and microenvironment. To complicate matters further, stromal cells themselves can be polarized into pro- and anti-inflammatory states² resulting in different secretomes.

At a minimum, the authors should provide detailed information about the preparation of their "breast cancer stem cells", as these are not standard reagents.

Additionally, UC- and adipose-conditioned media have not been characterized for cytokine/chemokine content. This is relatively simple to do, as multiplexed kits are commercially available and would significantly contribute to a more mechanistic understanding of the data.

A minor consideration: wording such as "nowadays" and "on top of that" are rather colloquial for a scientific publication.

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Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Cancer cell biology, regenerative medicine, flow cytometry, cGMP cell therapy

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have

significant reservations, as outlined above.

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