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Rat stromal vascular fraction (rSVF) cells can be directly transduced to express rat TNF-related apoptosis inducing ligand (rTRAIL), and exert similar but less prominent effects compared to rat adipose derived stem cells (rADSC) – an *in vitro* study

Wiktor Pascal[1,A,E-F], Katarzyna Marcinkowska[1,B,D,F], Antoni Smoliński[1,B-D,F], Mateusz Gotowiec[1,2,B,D,F], Paweł Krzysztof Włodarski[3,A,E-F]

[1] *Department of Methodology, Medical University of Warsaw, Warsaw, Poland*
[2] *Doctoral School, Medical University of Warsaw, Warsaw, Poland*
[3] *Department of Histology and Embryology, Medical University of Warsaw, Warsaw, Poland*
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Abstract

Introduction and Objective. Breast cancer remains a major clinical challenge, with high recurrence rates and the need for safer, more targeted local therapies. Biologic brachytherapy using genetically modified mesenchymal cells offers a promising strategy for localized cytokine delivery. While adipose-derived stem cells (rADSC) have shown potential as carriers of rat TRAIL (rTRAIL), their clinical translation is limited by the need for *ex vivo* expansion and concerns over tumour-promoting effects.

Materials and Method. The study investigated whether freshly isolated rat stromal vascular fraction (rSVF) cells could be directly transduced with a lentiviral vector to express rTRAIL. Their anticancer activity was compared to rADSC *in vitro* using co-culture with rat mammary gland cancer cells. The expression was also compared of several markers (CD90, CD44, CD29, CD105, VEGF2, CD31, CD45) between rSVF and rADSC.

Results. There were no significant differences in the expression of markers between rSVF and rADSC. Both cell types successfully expressed rTRAIL, with rTRAIL-rSVF producing approximately 50% of the TRAIL protein levels observed in rTRAIL-rADSC. In functional assays, rSVF-derived supernatants and co-cultures reduced breast cancer cell viability and colony formation, but the inhibitory effect was approximately 30–50% lower than that of rADSC, depending on the assay and cancer cell line used.

Conclusions. These findings confirm that rSVF can serve as a transducible and partially effective alternative for rTRAIL delivery and may be valuable for rapid, autologous therapies where culture expansion is not feasible. Further optimization and *in vitro* validation are needed.

Key words

breast cancer, *in vitro*, rat model, biologic brachytherapy

INTRODUCTION

Breast cancer remains the most frequently diagnosed malignancy worldwide. Despite the introduction of effective surgical and adjuvant therapies, its recurrence following mastectomy continues to pose a significant clinical challenge, with distant recurrence being about 13.5 % within three years in patients classified as *ypT0* following treatment [1]. Adjuvant treatments, such as chemotherapy and radiotherapy, though effective, are associated with systemic toxicity and local tissue complications, creating a growing demand for more targeted, locally confined therapeutic options.

✉ Address for correspondence: Wiktor Pascal, Department of Methodology, Medical University of Warsaw, Banacha 1b, 02-091 Warsaw, Poland
E-mail: wiktor.paskal@wum.edu.pl

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Biologic brachytherapy has emerged as a promising strategy that combines gene therapy with surgical approaches to deliver therapeutic molecules – most notably cytokines and proteins – directly to specific tissue compartments. This method utilizes local gene delivery via cellular or viral vectors to tissue fragments such as surgical flaps, thereby enhancing expression of therapeutic agents at the tumour site while minimizing systemic exposure [2]. Among the candidate molecules for such localized gene therapy, tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) has shown substantial promise due to its ability to induce apoptosis selectively in tumour cells through interaction with death receptors DR4 and DR5, sparing normal tissues [3]. However, breast cancer cells display variable sensitivity to TRAIL-mediated apoptosis [4]. Such TRAIL-resistance has been associated with reduction of DR4 and DR5, which

occurs via constitutive endocytosis [5] in some of the breast cancer cell lines. What is more, overexpression of some of the downstream regulators, including c-FLIP interferes with TRAIL-DR4/DR5 complexes, leading to reduced TRAIL sensitivity [6]. Finally, the expression of anti-apoptotic proteins, primarily Bcl-2 and Bcl-Xl, can limit TRAIL-induced apoptosis [7]. Multiple resistance mechanisms underscore the importance of TRAIL regulation, which is still not fully understood.

Mesenchymal stem cells (MSC), particularly adipose-derived stem cells (ADSC), have gained attention as vehicles for cancer-targeted gene delivery. In addition to their regenerative capabilities, MSCs display natural tumour-homing properties, allowing for targeted delivery of therapeutic genes to tumor environments [8, 9]. Previous studies have shown that TRAIL-expressing MSCs can suppress tumour growth in various models, including glioblastoma, hepatocellular carcinoma, and melanoma [10–14].

Despite their advantages, the clinical translation of ADSC-based therapies is hindered by the need for *ex-vivo* expansion, and growing evidence suggesting that under specific conditions, ADSC may support tumour progression. Several studies have reported that ADSC can enhance tumoursphere formation and contribute to recurrence via paracrine signaling, including activation of the HGF/c-Met pathway [15–17]. These conflicting findings emphasize the need for alternative cellular platforms that retain therapeutic potential while offering improved safety and clinical feasibility.

One such alternative is the stromal vascular fraction (SVF), a heterogeneous population of cells derived from adipose tissue that includes ADSC, endothelial cells, pericytes, and immune cells. Unlike ADSC, SVF can be isolated rapidly without *in vitro* expansion, making it more suitable for intraoperative or same-day therapeutic applications. While SVF has shown regenerative capabilities in tissue engineering, its role in cancer-targeted gene therapy remains largely unexplored. Assessing the potential of SVF cells as vectors of biologic brachytherapy is an important step for the successful translation of this research approach. As this study focused on an *in-vitro* assessment of SVF for the delivery of anticancer molecules, further *in-vivo* investigations are required to assess the effectiveness of SVF-based biologic brachytherapy in animal cancer models.

OBJECTIVE

The present study aimed to investigate whether freshly isolated rat SVF (rSVF) cells can be directly transduced with rTRAIL, and whether they exert comparable anti-tumour effects to rADSC. By comparing expression efficiency, apoptotic potency, and tumour-cell targeting *in vitro,* evaluation was sought of the potential of SVF as a readily available cellular vehicle for biologic brachytherapy. The findings may support future translational strategies of utilizing SVF in personalized, minimally invasive cancer treatments.

MATERIALS AND METHOD

Cell Culture. The rat adenocarcinoma mammary gland cell lines used in this study included RBA (CLR-1747, ATCC, Manassas, VA, USA) and HH-16.cl.4 (ACC 358, Leibniz-Institut DSMZ, Braunschweig, Germany). Cell cultures were maintained according to the respective supplier's protocols. RBA cells were cultivated in DMEM + Glutamax medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), while HH-16.cl.4 cells were maintained in RPMI 1640 medium (Gibco, Thermo Fisher Scientific). Each medium was supplemented with 10% foetal bovine serum (FBS; Sigma-Aldrich, Burlington, MA, USA) and a penicillin-streptomycin mixture (Gibco, Thermo Fisher Scientific, Waltham, MA, USA). Rat adipose-derived stem cells (ADSCs; 10RA-001), isolated from inguinal white adipose tissue were procured from iXCells Biotechnologies (San Diego, CA, USA) and cultured in DMEM/F12 + Glutamax medium supplemented with 10% FBS and an antibiotic mixture. SVF cells were kept in similar conditions, with the isolation method described below. The human embryonic kidney cell line Lenti-X 293T (Cat. No. 632180, Takara Bio, Clontech, Mountain View, CA, USA) was cultured in DMEM + Glutamax medium supplemented with 10% FBS and penicillin-streptomycin. All cell lines were screened for mycoplasma contamination using the LookOut® Mycoplasma PCR Detection Kit (Sigma-Aldrich) and confirmed to be mycoplasma-negative (Fig. S2).

SVF cells isolation The SVF isolation protocol used was based on a modified protocol by Kassem et al. [18]. First, epididymal fat pads were derived from male Sprague-Dawley rat and placed in Petri dishes containing phosphate-buffered saline (PBS, Gibco, Thermo Fisher Scientific, Waltham, MA, USA). Afterwards, fat tissue was minced and resuspended in 0.1% collagenase type 1 solution (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) and incubated in a shaking water bath (37 °C, 80 rpm, for 45 min). Later, it was centrifuged for 5 minutes at 300 × g and following decantation, the SVF pellet was resuspended in BSA solution (Sigma-Aldrich, Burlington, MA, USA) and centrifuged for 5 minutes at 300 × g. Afterwards, isolated SVF cells were suspended in complete media as described above and cultured in 25 cm2 flasks.

Plasmid cloning and lentiviral transduction of rADSCs. Total RNA was extracted from the kidneys of female Sprague-Dawley rats using the RNeasy Mini Kit (Qiagen, Hilden, Germany), and RNA integrity confirmed by A260/A280 absorbance ratio (≥ 1.8) using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific). Complementary DNA (cDNA) synthesis was performed using a GoScript reverse transcriptase (Promega, Woods Hollow, WI, USA). The full-length rat TRAIL gene, tagged with an N-terminal myc epitope and flanked by sequences for restriction site reconstruction, was amplified from cDNA using the CloneAmp HiFi DNA Polymerase (Takara Bio, Clontech). The primers used were as follows: forward primer: 5′-TATTTCCG- GTGAATTCATGGAGCAG-AAACTCA-TCTCTGAAGAGGATCTG-CCTTCCACCGG-GAAC-3′, reverse primer: 5′-GAGAGGGGCGGGATCCCTAGT-TAATTAAAAAGGCTCCAAAGAAACTGG-3′. The Plvx-CMV-IRES-ZsGreen1 plasmid, containing the ZsGreen1 fluorescent marker, was a gift from Professor Dominika Nowis at the Laboratory of Experimental Medicine of the Medical University in Warsaw, Poland. The plasmid was linearized using BamH1 HF and EcoR1 restriction enzymes (Thermo Fisher Scientific and New England Biolabs, respectively) and subsequently ligated with the TRAIL insert

using the InFusion HD Cloning Kit (Takara Bio). Plasmids were extracted using the MidiPrep Kit (Qiagen) and validated through backbone colony PCR, restriction enzyme digestion, and Sanger sequencing. Lentiviral particles were produced using a fourth-generation lentiviral packaging system (VSV-G envelope, Takara Bio). The lentiviral supernatants were concentrated ten-fold using the Lenti-X Concentrator Kit (Takara Bio). Titers were quantified using the p24 assay (Lenti-X Go-Stix, Takara Bio). rADSCs were transduced in antibiotic-free medium supplemented with polybrene (8 µg/mL; Thermo Fisher Scientific) and lentiviral particles encoding either the TRAIL gene or an empty control vector for 24 hours. To enhance transduction efficiency, cells were centrifuged at 560 × g for 1 hour. After 24 hours, the medium was replaced with fresh culture medium. Transduction efficiency was evaluated via fluorescence microscopy (PALM Robo, Zeiss, Oberkochen, Germany) at 24, 48, and 72 hours post-transduction, and further assessed up to 21 days.

RNA isolation, quantitative PCR. Total RNA was isolated from confluent cell cultures using the miRNeasy Kit (Qiagen). RNA quality (A260/A280 ≥ 1.8) was confirmed using a Nanodrop 2000 spectrophotometer. cDNA synthesis was performed using GoScript reverse transcriptase (Promega). The primers used were as follows: GAPDH_F: TGGGAAGCTGGTCATCAAC, GAPDH_R: GCATCACCCCATTTGATGTT, THY1_F: AGCTATTGGCACCATGAACC, THY1_R: GCTGATCACCCTCTGTCCTC, CD44_F: CCCTGGCCACCAGTGATG, CD44_R: TCCCACTTGAGTGTCCAGCTAA, ITGB1_F: ACAAGAGTGCCGTGACAACT, ITGB1_R: CTTCCGCACGCATCATTGAG, CD105_F: TCCTGACCTGTCTGGCAAAG, CD105_R: CTAGGGGCACGTGTGTGAGA, CD31_F: AACAGCCATTACGACTCCCAG, CD31_R: GGGAGCCTTCCGTTCTCTTG, CD45_F: GTCTTTGTCACAGGGCAAGG, CD45_R: AGTGTGGTGAGGTCAGCTTG, VEGF2_F: ATGTGGGGAAGGAGTTTGGA, VEGF2_R: GTTTGGGGCCTTGAGAGAGA.

ELISA for TRAIL quantification. Transduced and non-transduced ADSCs were harvested 72 hours post-transduction and subjected to 3 freeze-thaw cycles. TRAIL concentration was determined using the Rat TRAIL/TNFSF10 Quantikine ELISA Kit (R&D Systems, Minneapolis, MN, USA), and results were normalized to pg/mg per 100,000 cells.

Proliferation assay. Cancer cells (5120/well) were seeded in 96-well plates in an appropriate amount of cell medium, and 24 hours later were treated with 72-hour post-transduction supernatants gathered from rADSC. Cell proliferation was assessed using the CellTiter 96 AQueous One Solution Assay (Promega, Madison, WI, USA) after another 24 hours, and absorbance at 490 nm was measured.

Indirect co-culture of breast cancer cells and rADSCs. Cancer cells (200 cells/well) were seeded onto the lower compartments of 24-well plates and transduced rADSCs (10,000 cells/insert) were seeded onto 3 µm Transwell inserts, similarly to Wu et al. [19]. The cell medium was changed in the lower chamber every 3 days. After 9 days, cancer cell numbers on the bottom of the wells were evaluated using crystal violet staining.

Statistical analysis. Statistical analyses were performed using GraphPad Prism 10.3.1 (GraphPad Prism, San Diego, CA, USA). Significance was set at $p \leq 0.05$. One-way ANOVA was used for single-factor comparisons, and two-way ANOVA with Dunnett's multiple comparisons test was applied for multi-factorial analyses. The SAMPL guidelines for reporting statistical analysis were followed [20].

RESULTS

Isolation of SVF cells and determination of their similarities to rat ADSC. SVF cells were successfully isolated from rat epididymal fat pads and cultured for a period of 8 days (Fig. 1A). Initially, cells appeared round and unattached, but gradually adopted a spindle-shaped, adherent morphology. This shows that although the initial population of SVF cells was heterogeneous, over time the most proliferating cells were of mesenchymal origin.

To further determine the similarity between the isolated, cultured SVF cells, they were compared to phenotypically confirmed rat ADSC. To do this, focus was concentrated on a selection of 7 markers of adipose-derived stem cells, according to Mildmay-White and Khan [21]. Those markers included positive ones: CD90, CD44, CD29, CD105 and VEGF2 and negative ones: CD31 and CD45. The expression was compared of markers of mRNA levels, grouping the mean ΔCt mRNA expression values relative to glycerol-3-phosphate dehydrogenase (GAPDH), according to the predefined 'high vs. low expression pattern boundary' ($\Delta Ct \approx 8$). The results indicated extensive resemblance between isolated SVF cells and ADSC in terms of preselected markers (Fig. 1B). Some of the genes reached a significant difference (multiple T-test, $p \leq 0.05$), but among adjusted values only CD29 reached a significantly increased expression in SVF cells, compared to ADSC ($q = 0.018850$), and both positive and negative markers followed the established 'high vs. low' expression patterns, with CD90, CD44, CD29, CD105 and VEGF2 being detected in both populations at a high level ($\Delta Ct < 8$) and CD31 and CD45 not being detected, or at levels close to detection range ($\Delta Ct = 20$). ADSC populations demonstrated a higher relative expression of CD90, CD44, CD105 and CD45, compared to SVF cells. Conversely, SVF populations exhibited a higher relative expression of CD29 and VEGF2.

SVF cells can be directly transduced after isolation, however, the efficiency of target gene production may be lower compared to ADSC. Following the determination of the properties of isolated SVF cells, determination was sought of the transduction potential of this cell population to produce rat tumour-necrosis-factor(TNF)-Related Apoptosis Inducing Ligand (TRAIL), and compare it to the potential of rat ADSC. Therefore, two 2 vectors were used: original pLVX-CMV-IRES-ZsGreen1 plasmid (8204 base pairs), shortened to *Zsgreen1 fplasmid*, which acted as sham, and the modified pLVX-CMV-mycTRAIL-IRES-ZsGreen1 plasmid (9,054 base pairs), shortened to *mycTRAIL plasmid* containing the myc-tagged target transgene (Fig. 2A, 2B). Both plasmids were used for lentivirus production, and successfully transduced both cell populations (Fig. 2C, 2D). Differences were noticed

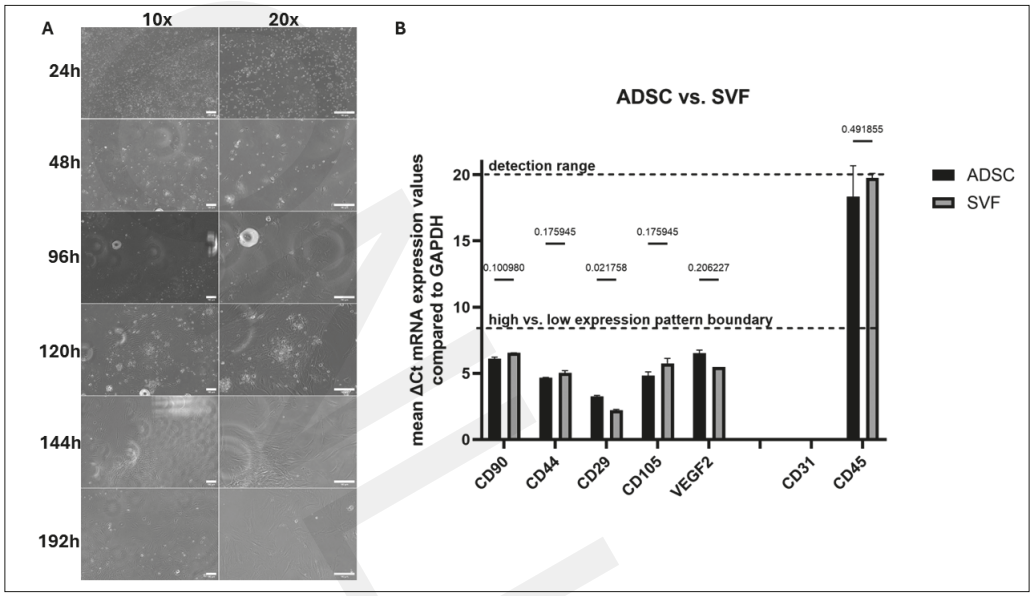

Figure 1. Determination of morphology of isolated rat SVF cells overtime and similarity to phenotypically confirmed rat ADSC. (A) Light microscope images showing the morphology of isolated SVF cells overtime from 24h after isolation, up to 7 days when they were passaged. The scale bar represents 100 um. (B) Differences in ADSC markers (CD90, CD44, CD29, CD105, CD31, CD45, VEGF2) expression between rat SVF cells and rADSC. The experiment was repeated independently two times in technical triplicates. Multiple t-test with false discovery rate (FDR) and two-stage step-up approach (Benjamini, Krieger, and Yekutieli), exact FDR-adjusted q-values presented for each of the markers

in transduction efficiency depending on the multiplicity of infection (MOI) used in both vectors, with similar efficacy (measured as semi-quantitative fluorescence intensity) of MOI=100 and MOI=160, and a lower efficacy when using MOI=30 in both cell populations. No major differences were noticed between cell populations; however, the fluorescence intensity was generally higher in SVF cells, compared to ADSC, especially in the vector containing the desired transgene (Fig. 2D). Finally, the concentration of TRAIL in different cell lysates were determined, which showed a statistically significant increase in mycTRAIL-transduced ADSC, compared to both sham-transduced and negative control ADSC (one-way ANOVA; $p \leq 0.05$) reaching a mean concentration of 50.9pg per 100,000 cells, compared to 13.6pg and 14.5pg per 100,000 cells in both control groups. On the other hand, the concentration of TRAIL in mycTRAIL-transduced SVF cells was lower, not reaching a statistically significant difference (one-way ANOVA, $p \geq 0.05$) compared to sham-transduced and negative control SVF cells, reaching a mean concentration of 24.8pg per 100,000 cells, compared to 16.9pg and 16.0pg per 100,000 cells in both control groups.

Effects of TRAIL-transduced SVF cells and ADSC on mammary gland cancer cells. After establishing the efficacy of the transduction of SVF cells and ADSC, the aim was to determine the effectiveness of their anti-tumour effects on rat mammary gland cancer cells, using two 2 cell lines – HH-16.cl.4 – a metastatic cell line, with HER2+ features [22] and RBA – an estrogene receptor negative *in-situ* derived cell line. First, cell supernatants were used, however, no major reduction in the proliferation of cancer cells was observed, with statistically insignificant growth inhibition of cancer cells after a 24-hour exposure to mycTRAIL-SVF cells' supernatants (Fig. 3A, 3B).

Afterwards, the aim was to determine the direct effects of SVF cells and ADSC on tumour cells and therefore an insert co-culture was used (Fig. 3C, 3D) to estimate the long-term effects of interactions between both cell groups. The results showed that genetically modified cells of both SVF and ADSC (both ZsGreen1 or mycTRAIL) had a moderate inhibitory effect on HH-16.cl.4 cells, with mean differences in colony area ranging from 5 – 15% percent (Fig. 3E). Moreover, mycTRAIL-ADSC were associated with a more pronounced reduction in colony area (%) compared to their respective controls; however, these differences did not reach statistical significance (one-way ANOVA, $p \geq 0.05$). For RBA cancer cells (Fig. 3F), a more notable decrease in cell proliferation was observed in genetically modified groups compared to non-modified controls. However, the results also did not reach a level of statistical significance (one-way ANOVA, $p \geq 0.05$). The reduction of cancer cell proliferation was strongest for cells exposed to Zsgreen1-ADSC and mycTRAIL-ADSC. In contrast, the mycTRAIL-SVF condition exhibited more modest inhibitory effects.

DISCUSSION

The study investigated the feasibility of using freshly isolated rat stromal vascular fraction (rSVF) cells as a direct vehicle for rTRAIL delivery, and compared their performance with cultured rat adipose-derived stem cells (rADSC). The results obtained demonstrate that although rSVF cells can be efficiently lentivirally transduced to express rTRAIL and exert mild anti-**tumour** effects *in vitro,* their impact on cancer cell viability and proliferation was consistently less pronounced compared to rADSC-based therapy. Similar results regarding TRAIL-transduced ADSC have already been confirmed using human ADSC against various cell lines as well as animal models [10,14,23]. However, none of the studies considered using directly extracted SVF cells, which is crucial for potential clinical translation.

Importantly, rSVF cells preserved their morphological heterogeneity and tolerated the transduction protocol

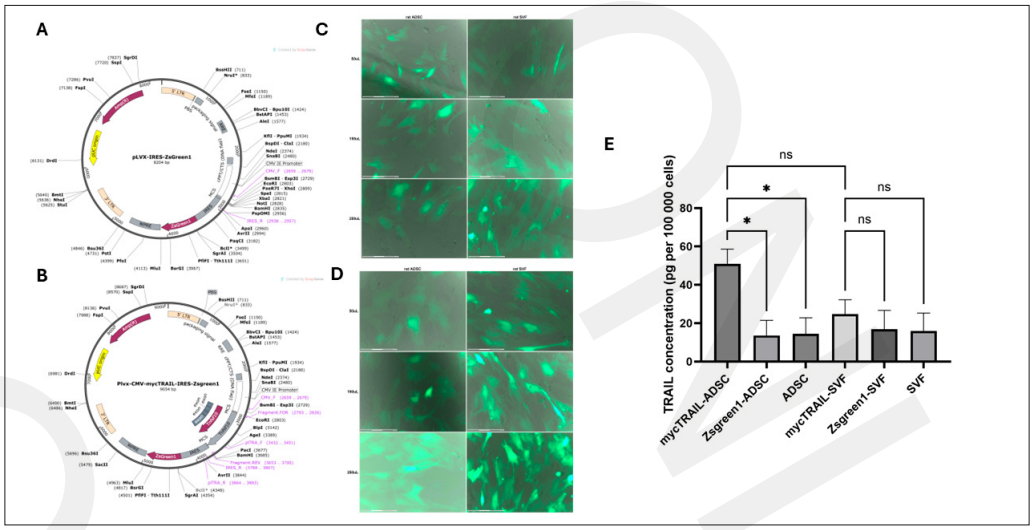

Figure 2. Rat SVF cells can be transduced after isolation, however the efficacy of transduction may be lower compared to ADSC. (A) Plvx-CMV-IRES-Zsgreen1 plasmid used for lentiviral transduction. (B) Modified Plvx-CMV-IRES-Zsgreen1 plasmid with cloned myc-TRAIL protein. (C) Fluorescence microscope image of Plvx-CMV-IRES-Zsgreen1-transduced rADSC and rat SVF cells at 72h post-transduction transduced with varying amounts of concentrated viral supernatant from transfected HEK293T cells (50ul, 150ul or 250ul concentrated supernatant, equivalent to MOI=30, 100, 160), 20x combined fluorescence and inverted image. The scalebar represents 150um. (D) Plvx-CMV-mycTRAIL-IRES-Zsgreen1-transduced rADSC and rat SVF cells at 72h post-transduction transduced with varying amounts of concentrated viral supernatant from transfected HEK293T cells (50ul, 150ul or 250ul concentrated supernatant, equivalent to MOI=30, 100, 160), 20x, combined fluorescence and inverted image. The scalebar represents 150um. (E) rADSC and rat SVF cells were transduced using 50, 150 and 250ul myc-TRAIL lentivirus or Zsgreen1 lentivirus and at t=72h samples were pooled and around 100 000 cells were collected and further frozen and thawed three times and an ELISA for rat TRAIL was run in duplicates in two independent experiments. The results were estimated based on the standards within normal range. One-way ANOVA was used to determine statistical significance between the groups. ns – p≥0.05, * - p≤0.05. Results presented as pg per 100 000 cells with standard error of mean (SEM).

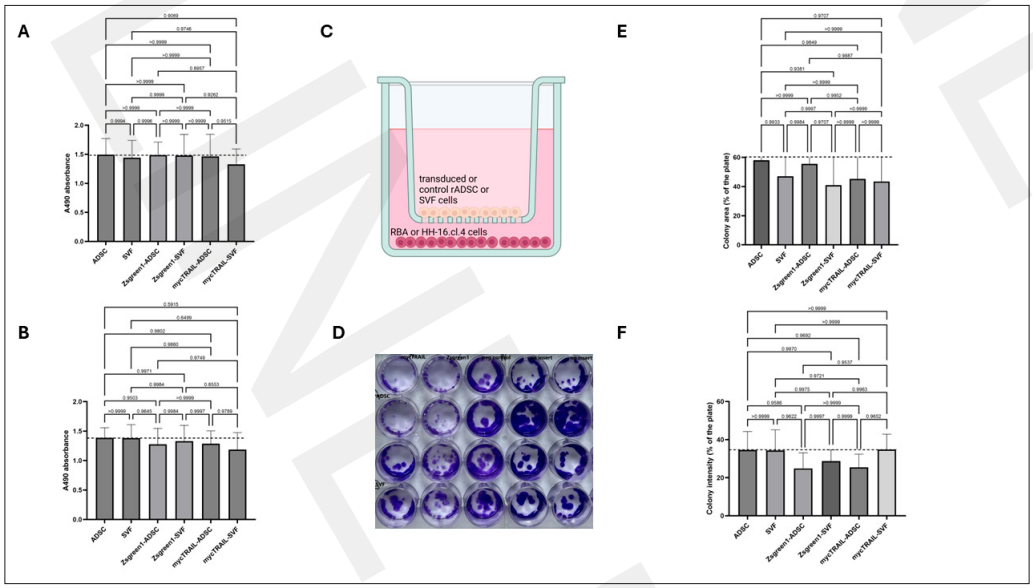

Figure 3. Both SVF cells and ADSC producing TRAIL moderately limit the growth of mammary gland cancer cells. Proliferation assay of HH-16.cl.4 (A) and RBA (B) rat breast cancer cells exposed to supernatant collected from transduced rADSC or SVF cells after 24 hours treatment (quadruplicates, experiment was repeated two times). One-way ANOVA was used to determine statistical significance between the groups. (C) Schematic picture of in-direct co-culture of 72h transduced rADSC/SVF cells and breast cancer cells and the sample picture of plate after Crystal Violet staining of lower chamber after 9 days of rADSC/SVF cells co-culture (D) The results of co-culture with HH-16.cl.4 cells presented as colony area (%) (E) and RBA cells presented as colony intensity (%) (F). One-way ANOVA was used to determine statistical significance between the groups

well. Transgene expression in rSVF was confirmed at both fluorescence and protein levels, albeit at lower concentrations than in rADSC. This result suggests that although both cell types can be genetically modified to deliver rTRAIL, intrinsic differences in cellular composition and transduction susceptibility likely account for the disparities in protein output and downstream biological effects.

The use of an indirect co-culture model in the study allowed assessment of the soluble fraction of rTRAIL activity, independent of physical contact between effector and target cells. However, this design did not allow evaluation of membrane-bound TRAIL activity, which is known to be more effective in some contexts [24]. Previous studies using direct co-culture have demonstrated the importance of cell–

cell proximity and membrane-bound ligand presentation in achieving optimal apoptotic effects. Future experiments will include direct contact assays to better delineate these mechanisms and optimize therapeutic design accordingly.

Functionally, both cell types demonstrated the ability to reduce proliferation and colony formation of TRAIL-susceptible rat mammary gland cancer cell lines. However, the response was modest and limited to specific cancer cell types. These findings reinforce the idea that breast cancer cells exhibit heterogeneous sensitivity to TRAIL-induced apoptosis, potentially due to differences in death receptor availability, decoy receptor expression, or the tumour microenvironment [25].

The observed differential response to TRAIL treatment between HH-16.cl.4 and RBA cells likely reflects intrinsic differences in TRAIL receptor expression, internalization, or apoptotic signalling pathway activation. Previous studies have shown that TRAIL resistance can arise from downregulation or endocytosis of death receptors (DR4/DR5), upregulation of decoy receptors, or anti-apoptotic regulators such as c-FLIP [26]. This supports the interpretation in the current study that RBA cells possess a more TRAIL-sensitive phenotype, while HH-16.cl.4 cells may harbour intrinsic or acquired resistance mechanisms.

Interestingly, the lower efficacy of rSVF in suppressing cancer cell growth may be attributable not only to lower TRAIL expression, but also to the presence of inhibitory subpopulations within SVF. This is supported by the multipotent and immunomodulatory nature of SVF, which includes endothelial progenitors, immune cells, and pre-adipocytes, some of which may antagonize TRAIL-induced effects, or promote survival signalling [27]. There is still no consensus on the overall safety of SVF augmentation for surgical breast reconstruction in cancer patients; however, most preclinical, as well as clinical studies confirmed that there were no risks of cancer progression after such treatment [28]. On the other hand, *in vitro* results have shown that ASC in co-culture with breast cancer cells could promote proliferation, migration and invasion [29]. Unfortunately, most of the studies focus only on a specific subpopulation of SVF, i.e. ADSC, therefore a limited amount of evidence regarding the other subpopulations is available. Interestingly, a recent study by Trevor et al. [30] shows that even following radiotherapy, SVF from irradiated breast tissue can still be considered for improving wound healing, comparable to SVF from healthy individuals, pointing towards the safety of SVF-based therapies.

When comparing rSVF and rADSC as cellular vehicles for rTRAIL delivery, each presents distinct advantages and limitations. rADSCs demonstrated higher transduction efficiency, greater rTRAIL protein production, and more pronounced anti-tumour effects in vitro. These results are consistent with their homogeneous stem cell phenotype and superior secretory capacity following culture expansion. However, rADSC-based therapies require time-consuming and resource-intensive in vitro culture, which may delay treatment initiation compared to rSVF.

In contrast, rSVF offers a unique set of translational advantages. It can be harvested and transduced intraoperatively, eliminating the need for *ex vivo* expansion. This feature enables the development of same-day, autologous cell therapies, particularly valuable in surgical oncology workflows where time-sensitive intraoperative interventions are needed. Furthermore, rSVF isolation is technically simpler and does not require Good Manufacturing Practice (GMP) -compliant expansion, reducing regulatory burden and cost. While its therapeutic efficacy *in vitro* was 30–50% lower compared to rADSC, this may be an acceptable trade-off in clinical settings prioritizing speed, safety, and logistical simplicity.

Potential application scenarios for rSVF-based TRAIL delivery include: (1) biologic brachytherapy in breast-conserving surgery or mastectomy, where SVF-transduced flaps or matrices could be used intraoperatively; (2) rapid autologous therapy for patients ineligible for delayed or culture-based cell therapies; and (3) combination approaches with other cytotoxic or immunomodulatory agents, where rSVF may serve as a supportive therapeutic vector.

From a translational perspective, the findings of the current study are promising. rSVF cells can be harvested intraoperatively without the need for culture expansion, making them an appealing candidate for rapid, personalized gene therapy strategies. Their slightly lower efficacy compared to rADSC may be compensated by practical advantages in clinical settings, particularly when used as part of biologic brachytherapy protocols [2]. However, further *in vivo* studies are essential to determine whether the reduced efficacy observed *in vitro* translates to clinically meaningful differences.

The present study was designed as an *in vitro* feasibility assessment of rSVF-based TRAIL delivery. While in vivo studies are essential for evaluating anti-tumour efficacy, biodistribution, and safety, early-stage *in vitro* work enables the optimization of gene delivery protocols and screening of therapeutic effects prior to animal experimentation. These findings provide a functional foundation for future *in vivo* validation in syngeneic or orthotopic breast cancer models.

Limitations of the study. First, although TRAIL expression was confirmed at the protein level by ELISA and qualitatively by fluorescence microscopy, no Western blotting was performed. ELISA was used as the method of choice due to its specificity for soluble TRAIL and its quantitative output, which was sufficient to compare expression levels between rADSC and rSVF.

Furthermore, the assays used focused on functional readouts (viability and colony formation) rather than direct apoptosis detection, which limits mechanistic interpretation. The observed difference between supernatant cytotoxicity and co-culture results suggests that cell–cell proximity is critical for TRAIL efficacy – a notion previously reported in other models using TRAIL-expressing stem cells [12].

Future studies will address these limitations using annexin V assays and caspase activation assays to further confirm apoptosis induction.

The reduced efficacy of rSVF compared to rADSC may reflect not only differences in TRAIL expression, but also the presence of inhibitory or non-contributory cell subsets within the SVF population. While gene expression of common surface markers (CD90, CD105, CD44, etc.), was assessed, it is acknowledged that a more detailed phenotypic analysis – including flow cytometry to quantify cell subset proportions – would be necessary to fully characterize SVF heterogeneity. Future studies should explore whether selective enrichment of specific subpopulations could enhance therapeutic efficacy and consistency.

CONCLUSIONS

In conclusion, while rADSC remains a more robust platform for rTRAIL-mediated cytotoxicity, rSVF represent a practical and transducible alternative for future personalized cancer therapies. Their application may be especially valuable in rapid autologous settings where time and resources preclude cell culture expansion. Further studies should explore optimizing transduction protocols and dissecting the role of individual SVF subpopulations to enhance therapeutic consistency and potency.

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