Research Paper

Effects of Mesenchymal Stem Cell-Conditioned Medium on Human Dermal Fibroblasts and Epidermoid Carcinoma Cells: Implications for Wound Healing

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ABSTRACT

Background: This investigation was directed to studying the impact of mesenchymal stem cells (MSCs) derived- conditioned medium (CM) in normal human dermal fibroblasts (HDF-a), and A431 epidermal carcinoma.

Methods: The cancerous and normal cell lines (A431 and HDF-a) were treated with four groups (DMEM, DMEM + FBS, CM, and CM + FBS) for 24, 48, and 72 hours. Then, the rate of proliferation and migration and TGF-β gene expression were examined using MTT assay, scratch assay, and real-time PCR respectively.

Results: Data showed that the MSCs-conditioned medium increased proliferation of HDF-a, whereas it had no significant effect on A431. Migration rate was faster in HDF-a than in A431 in scratch assay. The expression of TGF-β was up-regulated in HDF-a cell treated with conditioned media compared to the skin cancer cells.

Conclusion: This work suggests that MSCs derived- conditioned medium may play a role in skin wound healing by increasing proliferation and migration, along with alleviating TGF-β. Our findings show the interaction of A431 epidermoid carcinoma cells with MSC-conditioned medium is facing controversial concerns.

Keywords: Conditioned medium, Mesenchymal Stem Cells (MSCs), Human Dermal Fibroblast, A431 Epidermal Carcinoma

Introduction

Wound healing is a dynamic and tightly regulated process involving inflammation, tissue regeneration, and remodeling. Dermal fibroblasts play a pivotal role in this cascade by producing extracellular matrix (ECM) components and growth factors essential for tissue repair. However, in chronic wounds or compromised healing environments, fibroblast function is often impaired, necessitating novel therapeutic strategies to enhance regeneration [1]. Mesenchymal stem cells (MSCs), known for their multipotency and immunomodulatory properties, have emerged as promising candidates in regenerative medicine. Beyond their differentiation potential, exert significant paracrine effects through the secretion of bioactive molecules, collectively termed the secretome. These factors, including cytokines, growth factors, and extracellular vesicles, can be harvested in the form of conditioned medium (CM), offering a cell-free therapeutic alternative that circumvents the risks associated with direct cell transplantation [2, 3].

Adipose-derived MSCs (ADSCs), in particular, have demonstrated the ability to modulate fibroblast activity, promote ECM remodeling, and influence key signaling pathways such as transforming growth factor-beta (TGF-β), a central mediator in wound healing and fibrosis. However, the dual role of MSCs and their secretome in both tissue regeneration and tumor progression has raised concerns, especially regarding their interactions with cancer cells. Studies have shown that MSC-derived factors can influence tumor behavior by modulating inflammatory cytokines (e.g., IL-6, VEGF, TNF-α), matrix metalloproteinases (MMPs), and oncogenic pathways such as STAT3 [4, 5].

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Given these dualistic effects, it is critical to evaluate the safety and efficacy of MSC-CM in regenerative applications, particularly in contexts where cancerous cells may be present. In this study, we investigated the effects of MSC-conditioned medium on two distinct human cell lines: HDF-a dermal fibroblasts, representing normal skin cells involved in wound healing, and A431 epidermoid cells, serving as a model for skin cancer [6]. We assessed cell proliferation, migration, and TGF-β gene expression following treatment with various culture conditions, including MSC-CM with and without fetal bovine serum (FBS). Our findings aim to elucidate the regenerative potential of MSC-CM in promoting fibroblast function while evaluating its influence on tumor cell behavior. This dual analysis provides critical insights into the therapeutic promise and oncological safety of MSC-derived secretomes in skin wound healing applications [7].

Methods

Cell Culture and Collection of Conditioned Media

The human epidermoid carcinoma cell line (A431) and skin fibroblast cell line (HDF-a) were collected from the National Cell Bank of Iran (Pasteur Institute, Iran). The cells were cultured in DMEM medium containing 10% fetal bovine serum (FBS) plus 1% penicillin/streptomycin, and incubated at 37 ° C in 5% CO2 with 95% humidity. The MSC line was purchased from Royan Stem Cell Bank and cultured in the same conditions. To collect CM, cells were seeded into 6-well plates and incubated to achieve 70% confluence. After 72 hours, the conditioned cell culture media were collected, and the cells were washed with PBS to completely remove the media. Then the medium was centrifuged at 2000 RPM for 5 min, and filtered through a syringe filter to remove any dead cells or extra cell fragments. CMs were frozen at – 20°C until needed.

MTT Assay

The MTT assay was conducted to assess the effects of various culture media DMEM, DMEM supplemented with 10% fetal bovine serum (DMEM+FBS), mesenchymal stem cell-conditioned medium (CM), and a combination of CM with FBS (CM+FBS) on the viability of A431 and HDF cell lines. Cells were seeded into 96-well plates at a density of 1.5×10^4 cells per well in 200 µL of the respective medium and allowed to adhere overnight. Following incubation, cells were treated with the designated media for 24, 48, and 72 hours. At each time point, 10 µL of MTT solution (5 mg/mL, Sigma) was added to each well and incubated for 3 hours at 37 °C to facilitate the formation of formazan crystals. Subsequently, the medium was removed, and the crystals were solubilized by adding dimethyl sulfoxide (DMSO). Absorbance was measured at 570 nm using an ELISA microplate reader to quantify cell viability.

Cell Migration Assay

A431 and HDF cells were seeded into 6-well plates at a cell density of 15×10^4 cells/well in four different media: DMEM, DMEM+FBS, CM, and CM+FBS+DMEM. Cell Viability numbers were determined using 0.4% Trypan blue exclusion dye (Gibco, ThermoFisher Scientific, UK). The wells were scratched vertically with a 200 µL pipette tip. Finally, after in 3 separate times (24h, 48h, and 72h) incubation, the images of scratched fields were captured. The scratched areas were measured using the ImageJ 1.47 software.

Quantitative Reverse Transcriptase-polymerase Chain Reaction (qRT-PCR)

Transdifferentiated mesenchymal stem cells (MSCs) were characterized by reverse transcription polymerase chain reaction (RT-PCR). Total RNA was extracted using the RNeasy Plus Kit (RnxPlus, CinnaGen, Iran), and cDNA synthesis was performed with the PrimeScript First Strand cDNA Synthesis Kit (Kiagene Fanavar, Iran). Briefly, 1 µg of total RNA was reverse transcribed at 42 °C for 1 hour in a reaction mixture containing 500 µg/mL Oligo(dT), 10 mM deoxyribonucleotide triphosphates (dNTPs), 5× first-strand buffer, 0.1 M dithiothreitol (DTT), and 200 U/mL reverse transcriptase. The reaction was carried out using a Kyratec Super Cycler.

Quantitative real-time PCR (qRT-PCR) was performed using gene-specific primers and the KAPA SYBR FAST qPCR Kit (KAPA Biosystems). The thermal cycling conditions included an initial denaturation at 95 °C for 10 minutes, followed by 40 cycles of amplification (95 °C for 15 seconds, annealing at 64 °C for hTERT and 60 °C for GAPDH for 30 seconds, and extension at 72 °C for 30 seconds). Reactions were conducted in triplicate using an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, USA). GAPDH (Gene ID: 2597) was used as the endogenous control. Relative gene expression levels of TGF-β (Gene ID: 7040) were calculated using the $2^{\wedge-\Delta\Delta Ct}$ method. Negative controls (no template) were included in each run, and all reactions were performed in triplicate within the same 96-well plate.

Statistical Analysis

Experiments were performed in triplicate, and data sets are expressed as mean ± standard deviation (SD). Data were analyzed using the Student's t-test. Statistical significance is presented as $^*P < 0.05$.

Results

Doubling Time

Cell doubling times are critical parameters for optimizing treatment schedules and evaluating the regenerative potential of cell populations in wound healing. As shown in Fig. 1A and 1B, the doubling time for A431 was 48 hours, whereas HDF-a exhibited a faster proliferation rate with a doubling time of 24 hours.

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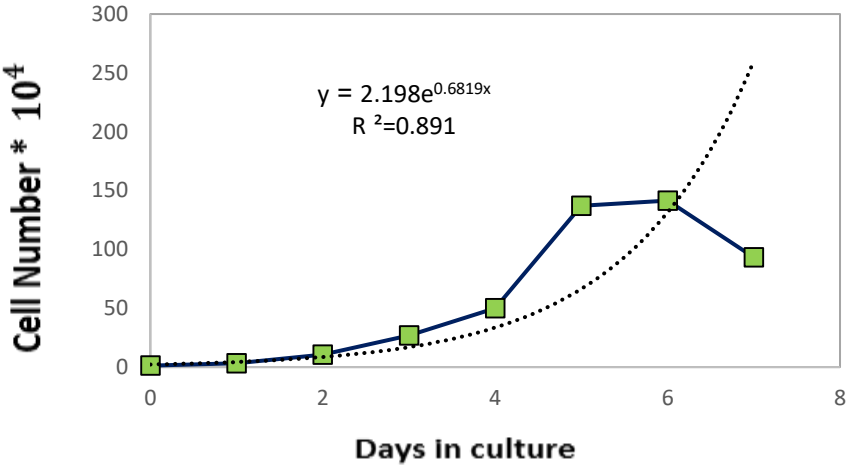
These differences in proliferative dynamics may influence their respective roles in re-epithelialization and dermal matrix remodeling during the wound repair process.



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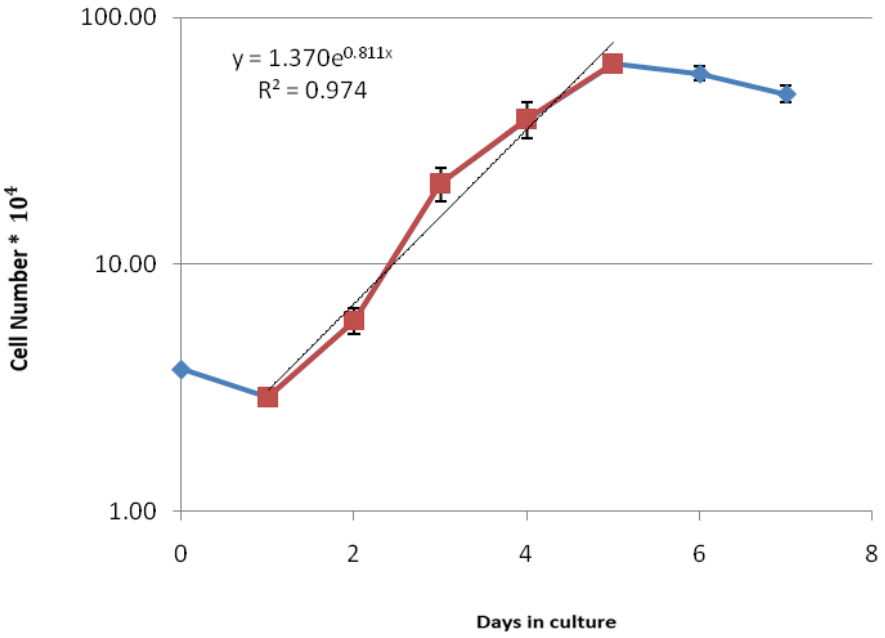

B

Figure.1. Average doubling time of A, A431 and B, HDF-a. Mean doubling time ± standard error of the mean (SEM, n=3) was reported.

Cell Proliferation Assay

We used the MTT test to measure cell viability. In this test, we examined A431 and HDF-a cells in four different environments: DMEM, DMEM + FBS, CM, CM + FBS. To have greater accuracy, MTT assay was performed three times for each medium in triplicate. The following diagrams are drawn according to the results obtained from the MTT test. The effects of MSC-CM on A431and HDF-a cells were evaluated at 24, 48, and 72 hours. As shown in Figure 2A, the viability of the HDF-a cells increased significantly.

Scratch Wound Assays

A431 (Fig. 3A) and HDF-a (Fig. 3B) scratch assays were done in the presence of DMEM, DMEM with FBS, MSC CM with and without FBS. There was a significantly increased rate of wound closure for both cells. In general, HDF-a fibroblasts migrate more rapidly than A431 cells.

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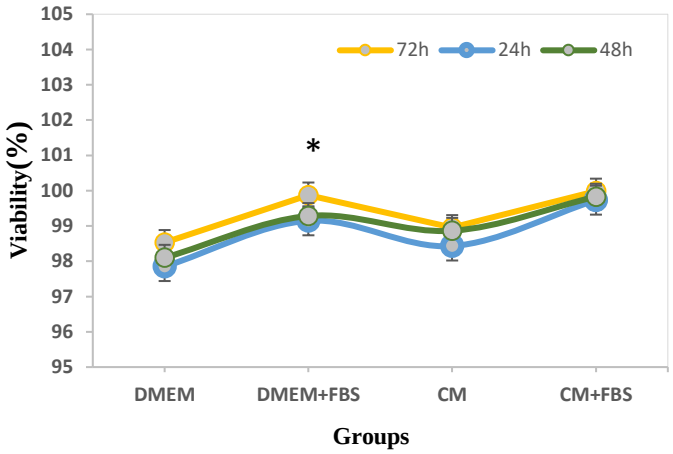

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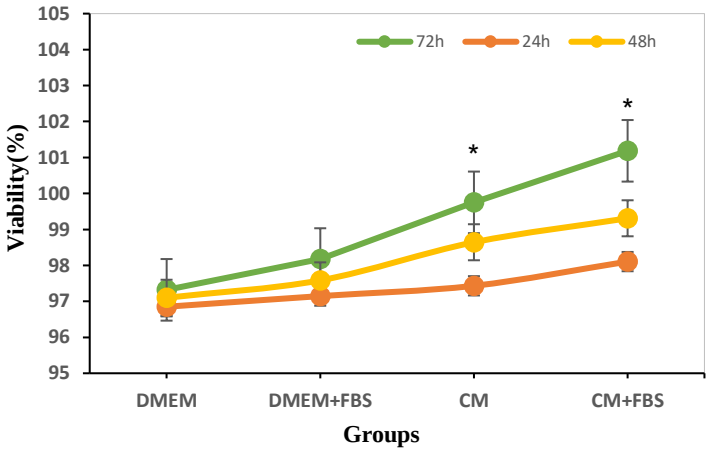

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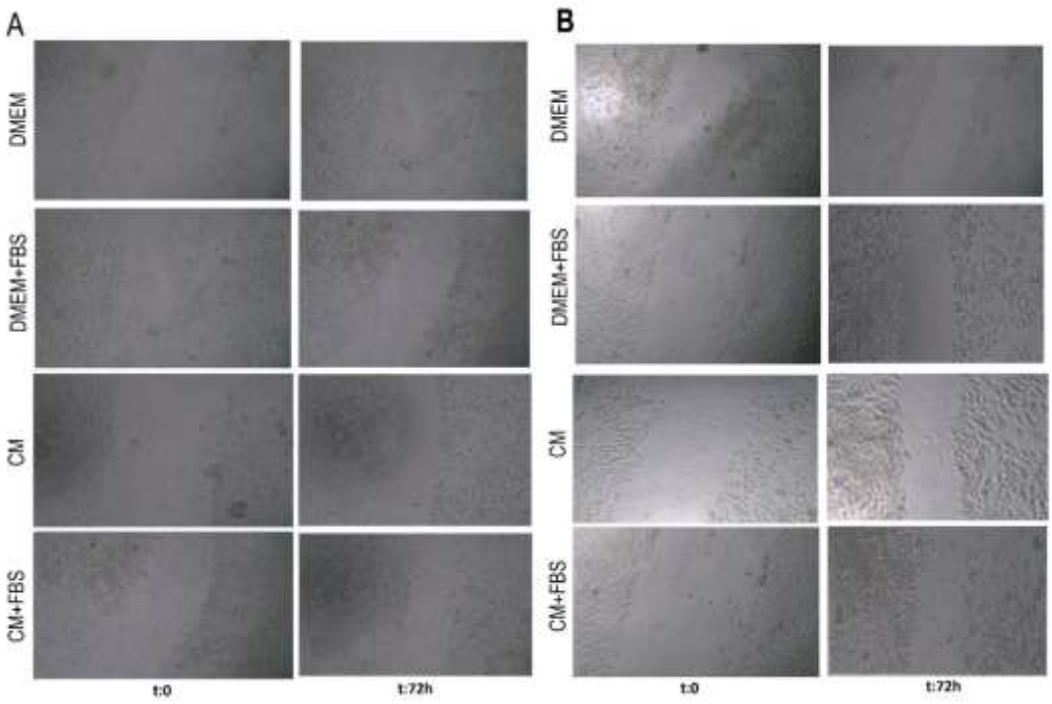
Figure 2. The effect of MSC-CM on the viability of A) A431 B) HDF-a. Cells were examined at 24, 48, and 72 hours and the cell viability was assessed using MTT assay and the data was shown as means ± SD from triplicate experiments. *p < 0.05.



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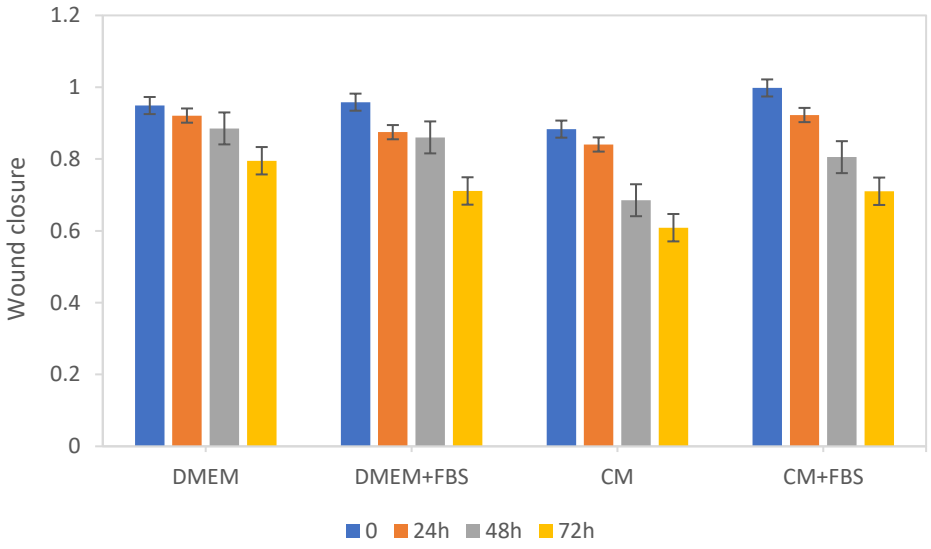

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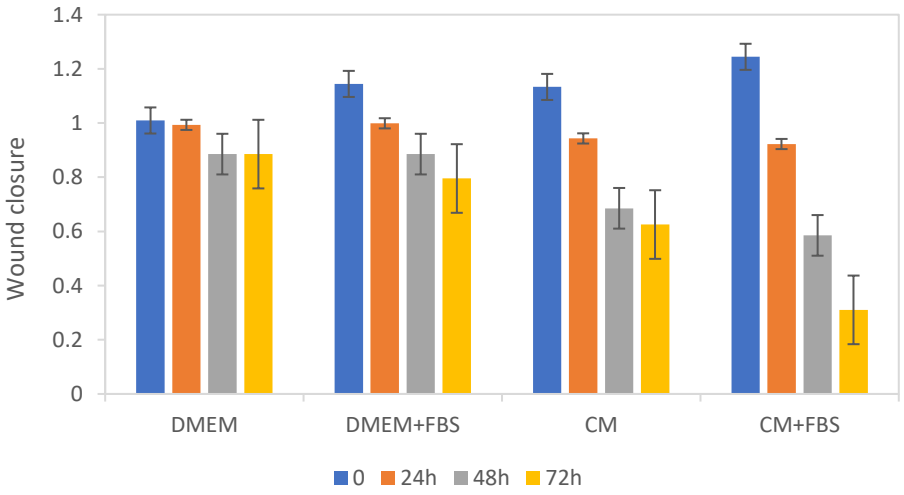

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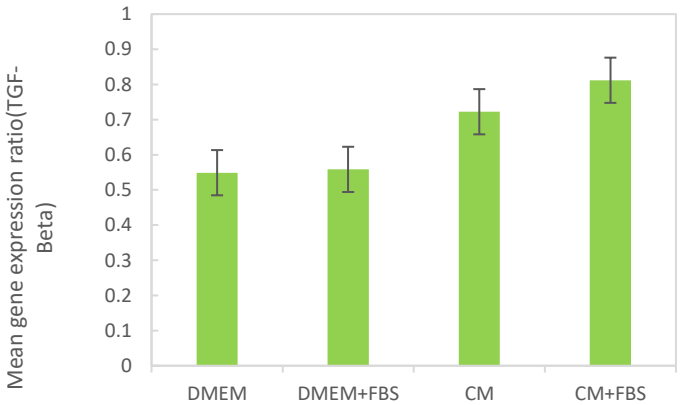
Figure 3. Measurement of A) A431 and B) HDF-a migration by in vitro scratch assay. The HDF dermal fibroblast migrates more rapidly than A431 in the presence of conditioned media



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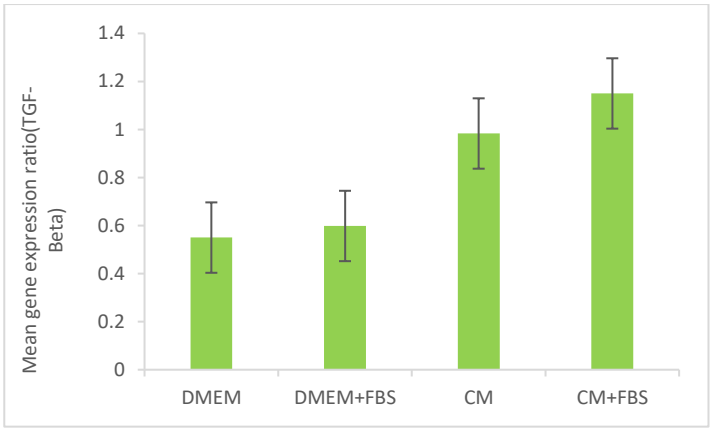

B

Figure 4. Relative expression analysis of TGF-β using qRT-PCR in A431 (A) and HDF-a (B). Elevated expression of TGF-β in cells treated with CM compared with DMEM and DMEM with FBS. Results are the mean ± SEM. Each test was repeated three times. * P < 0.05

Table 1. Primer sequence, and product length of GPPDH, and TGF-Beta

Genes and Primers	Sequence
TGF-Beta	
F Primer	5′TACCTGAACCCGTGTTGCTCTC3′
R Primer	5′GTTGCTGAGGTATCGCCAGGAA3′
GAPDH (RT-PCR) GAPDH (qRT –PCR)	
R Primer	5′GAGTCCTTCCACGATACC3′
F Primer	5′CCAGCCGAGCCACATCGCTC3′

Expression Evaluation of TGF-β in HDF and A431

Figure 4 A&B showed that the expression of TGF-β was increased in the cells that were treated with conditioned media in two different cell lines. The present results demonstrate a cell type–dependent regulation of TGF-β expression, with a modest increase in A431 epidermoid carcinoma cells and a marked upregulation in human dermal fibroblasts (HDF), particularly under conditioned media (CM) and CM supplemented with FBS.

Discussion

This study focused on comparing key characteristics of A431 carcinoma cells and HDF-a fibroblasts, including their proliferative capacity, migratory behavior, and response to paracrine signaling under various culture conditions. A particular emphasis was placed on exploring the potential wound healing benefits of mesenchymal stem cell-conditioned medium (MSC-CM). Analysis of doubling time revealed that HDF-a fibroblasts exhibited a significantly faster proliferation rate (24 hours) compared to A431 cells (48 hours), a behavior aligned with the physiological role of fibroblasts in tissue regeneration and their capacity for rapid growth during wound repair. This advantage in proliferation was further supported by MTT assays, which demonstrated increased viability in both cell types treated with MSC-CM, particularly when fetal bovine serum (FBS) was present. Notably, A431 and HDF-a cells showed enhanced cell survival metrics over 72 hours, suggesting that MSC-CM contains bioactive components that promote cell survival and proliferation through synergy with serum-derived growth factors. Scratch wound assays provided additional insights into cell functionality, revealing that HDF-a fibroblasts migrated more rapidly than A431 cells under all tested conditions, especially in the presence of MSC-CM. This heightened migratory behavior is essential for effective wound healing since it underpins extracellular matrix formation and tissue. Additionally, the treatment of both cell types with MSC-CM led to upregulated TGF-β expression, particularly in conditions enriched with FBS. TGF-β plays a pivotal role in regulating fibroblast activation, collagen production, and epithelial-to-mesenchymal transition processes. Its increased expression points to MSC-CM's ability to prime fibroblasts and epithelial cells' regenerative activities. Nevertheless, the responsiveness of A431 carcinoma cells to MSC-CM introduces critical considerations regarding the dual nature of MSC-derived factors. While these components can enhance fibroblast-driven wound repair by promoting proliferation, migration, and TGF-β signaling, they may also inadvertently support tumor cell growth and motility under certain contexts. Therefore, while the findings highlight MSC-CM's therapeutic promise in advancing fibroblast-mediated wound healing, additional studies are essential to thoroughly evaluate its safety profile. Particular attention should be given to minimizing potential risks related to tumor progression and optimizing its clinical application accordingly.

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Practical Implications

This study suggest that the conditioned media can modulate the behavior of both normal and malignant cells. These insights provide a foundation for designing targeted therapeutic strategies that facilitate wound repair while simultaneously inhibiting the proliferation of malignant cells in tumor-associated wounds

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Author's Contributions

Rezakhani N.: project administration, methodology, witting, and formal analysis. Keshmiri Neghab H.: project administration, writing, validation, and methodology. Soheilifar M.H.: conceptualization, review and editing.

Ethical Considerations

This study was conducted using established cell lines. As no human participants or animal subjects were involved in the experiments, institutional ethical approval was not required.

Conflicts of Interest

The authors declare no conflict of interest, financial or otherwise.

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