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Inhibition of Cervical Cancer Cell Migration by Human Wharton's Jelly Stem Cells

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ABSTRACT

Background: One of the most prevalent malignancies to strike women, both in Iran and globally, is cervical cancer. Metastasis, which is a significant cause of mortality, is one of the most significant pathological processes of this cancer. Therefore, preventing the migration of cancer cells may be a useful therapeutic approach. The aim of this work was to investigate the impact of conditioned media and human Wharton's jelly stem cells (hWJSCs) on the migration and growth of the cervical cancer cell line Hela as well as the in vitro mRNA expression of genes involved in metastasis.

Materials and Methods: After primary culture, cellular extract and conditioned medium of hWJSCs were prepared. The viability of cervical cancer cells was investigated by MTT assay after treatment with cellular extract and conditioned medium of hWJSCs. Moreover, the anti-migratory effects of cellular extract and conditioned medium of hWJSCs on the cervical cancer cells were evaluated by wound-healing migration assay. Finally, the mRNA expression of migration-related genes (*E-cadherin* and *vimentin*) was detected by real-time PCR.

Results: Our results indicated that the cellular extract and conditioned medium of hWJSCs (with 32% concentration) inhibited the proliferation of 100% and 20% of Hela cancer cells, respectively. In addition, the cellular extract and conditioned medium of hWJSCs significantly decreased morphological alteration and migration of the cancer cells. The cellular extract and conditioned medium of hWJSCs modified the expression of *vimentin* and *E-cadherin* genes to inhibit the cancer cell migration ($P<0.05$). However, the cellular extract indicated significantly profound inhibitory effects on the cervical cancer cells compared to the conditioned medium.

Conclusion: Our study demonstrated that the cellular extract and conditioned medium of hWJSCs inhibit the proliferation and migration of cervical cancer cells through the modification of migration-related gene expression. However, further in vitro and in vivo studies are required for more accurate results.

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Introduction

In women, cervical cancer is the fourth most frequent malignancy and the fourth leading cause of mortality worldwide. Each year, more than 500,000 women receive a cervical cancer diagnosis, with rates being higher in underdeveloped nations [1]. According to a recent study [2, 3], cervical cancer is the second most common disease in women and the second major cause of cancer-related death in Iran. According to the available data, cervical cancer is significantly influenced by the human papillomavirus (HPV), which is believed to be the cause of more than 80% of cases [4].

Currently, cervical cancer treatment includes a wide range of therapeutic techniques; however, its management is still challenging. The suppression of cancer cells through the induction of apoptosis is a crucial therapeutic technique in the management of many malignancies [5, 6]. Cervical cancer is associated with uncontrolled cell division and increased anti-apoptotic gene expression. The high death rate among those with various cancer types is a result of metastasis and tumor growth in remote regions [7, 8].

The evidence suggests that mesenchymal stem cells (MSCs) inhibit cancer cells by triggering the apoptotic pathway [9]. MSCs frequently exhibit therapeutic effects as a result of many released cytokines [10], growth hormones, extracellular matrix proteins, etc. [11, 12]. Human Wharton jelly stem cells (hWJSCs) are produced in the embryonic cord, which is generally destroyed after birth, and possess both embryonic and mesenchymal stem cell qualities [13]. The umbilical cord is encircled by Wharton jelly, a mucoid connective tissue. Recently, the anti-proliferative and inhibitory effects of hWJSCs on cancer cells were indicated [14, 15].

Due to the characteristics of MSCs and their anti-cancer effect, as well as the fact that cell therapy has fewer side effects than other treatments, like surgery and chemotherapy, the current study looked at the influence of hWJSCs on the proliferation and migration of Hela cervical cancer cells.

Materials and Methods

hWJSCs isolation and culture

We collected ten umbilical cord samples from pregnant women at the delivery time referred to Valiasr Hospital, Tabriz, Iran. The explant culture method was applied to isolate hWJSCs. Cell culture was performed using Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin antibiotic, and then incubated at 37°C with 96% humidity and 5% CO_2.

Preparation of the conditioned media for hWJSCs

The isolated hWJSCs were cultured in an FBS-free culture medium and allowed to grow up to 70-80% cell density. The supernatant (conditioned medium) was sterilized by a 0.22 µm filter, and stored at -20°C.

Preparation of the hWJSCs extract

The isolated hWJSCs were grown in complete culture media until they reached a cell density of 70–80%. Phosphate buffer saline (PBS) was used to wash the old culture media twice. The cells were lysed in 5 mL of protease inhibitor-containing RIPA lysis buffer (150 mM NaCl, 1.0% nonyl phenoxypolyethoxyethanol-NP40, 50 mM Tris-Cl, 1.0% sodium deoxycholate, and 0.1% SDS). A 0.22 µm pore size filter was used to sterilize the resulting suspension, and the supernatant (cellular extraction) was then stored at -20°C.

Hela cancer cell culture

From the Pasteur Institute in Tehran, Iran, the cervical cancer cell line Hela was obtained. The RPMI 1640 medium, which contains 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin antibiotic, was used to culture the cancer cells. The cells were kept at 37°C with 96% humidity and 5% CO_2 during this process.

Cancer cell viability assay

Cancer cells were seeded in a 96-well plate at a density of 15×10^3, followed by incubation overnight. Different concentrations of the conditioned media (16%, 24%, 32%, 48%, 56%, 64%, and 76%) and hWJSCs extract (2%, 4%, 8%, 16%, 24%, and 32%) were used to treat the cancer cells. Tetrazolium micro-culture (MTT) assay was used to assess the vitality of cancer cells after 72 hours of incubation. To achieve this, the old culture media was removed, and each well received 2 mg/mL of MTT solution in RPMI before being incubated for 4 hours. Dimethylsulfoxide (DMSO) was then added to each well and incubated once more for 30 minutes after the previous culture media had been discarded. An ELISA reader was used to quantify the optical density (OD) of dissolved formazan at 570 nm.

Table 1. Sequences of the used primers for the detection of metastasis-related gene expression

Gene	Primer Sequence	Products Size
Vimentin	F: GTGAATACCAAGACCTGCTCAATG R: ACCTGTCCATCTCTAGTTTCAACC	220 bp
E-cadherin	F: GACCAGGACTATGACTACTTGAAC R: GCTATACTAACTGCATCACTAACCA	224 bp
Beta-actin	F: TGCCCATCTACGAGGGGTATG R: CTCCTTAATGTCACGCACGATTTC	155 bp

Morphological alteration assay

The cancer cells were seeded in a 6-well plate at 2×10^5 cells/well density, and incubated overnight. The cancer cells were treated by conditioned medium and hWJSCs extract, and incubated for 48 hours. The morphological alteration was monitored using inverted light microscopy [14].

Migration assay

The cancer cells were seeded in a 6-well plate at 2×10^5 cells/well density, and incubated overnight. The center of each well was scratched with monolayer cells using pipette tips. The detached cells were removed by washing them with PBS. The cancer cells were treated with the conditioned medium and hWJSCs extract and then incubated for 72 hours. The migration rate of the cancer cells was measured by inverted light microscopy [14].

Gene expression assessment

The RNA was extracted using the TRIzol agent (Gibco, USA) in accordance with the manufacturer's instructions. The cDNA was produced using a specific cDNA synthesis kit (Yekta Tajhiz, Iran) and random hexamers. The expression of the genes encoding the migration-related proteins *vimentin* and *E-cadherin* was evaluated using real-time PCR. The used primers along with their descriptions are shown in Table 1. One microlitre of cDNA, 5 µL of master mix, 0.5 µL of forward primer, 0.5 µL of reverse primer, and 3 µL of deionized distilled water were used to conduct the PCR reaction in a total volume of 10 µL. The PCR condition also included a cycle of initial denaturation at 94˚C for 60 seconds, 40 cycles of denaturation at 94˚C for 20 seconds, 40 cycles of annealing and extension at 54˚C for 30 seconds, and 40 cycles of each of these temperatures for 30 seconds. β-actin (ACTB), a housekeeping gene, was used as an external control. The $2^{-\Delta\Delta Ct}$ (Livak) formula was used to calculate the results.

Results

Cytotoxic effects of hWJSCs

Our research established the time- and concentration-dependent nature of the cytotoxic effects of the conditioned media and hWJSCs extract. Compared to the conditioned medium, cancer cells treated with hWJSCs extract experienced a greater rate of cell death (Figure 1).

Morphologic alterations in cancer cells

Following treatment with hWJSCs conditioned media and extract, the cervical cancer cells showed some mor-

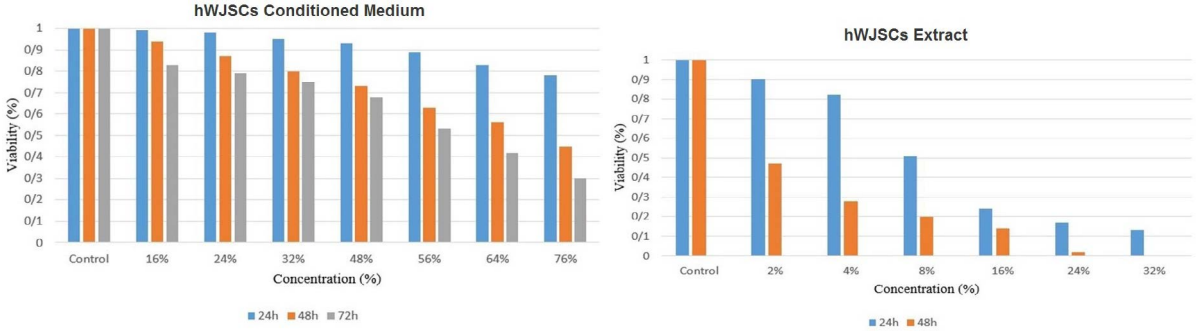

Figure 1. The viability of cervical cancer cells treated with the hWJSCs conditioned media and extract

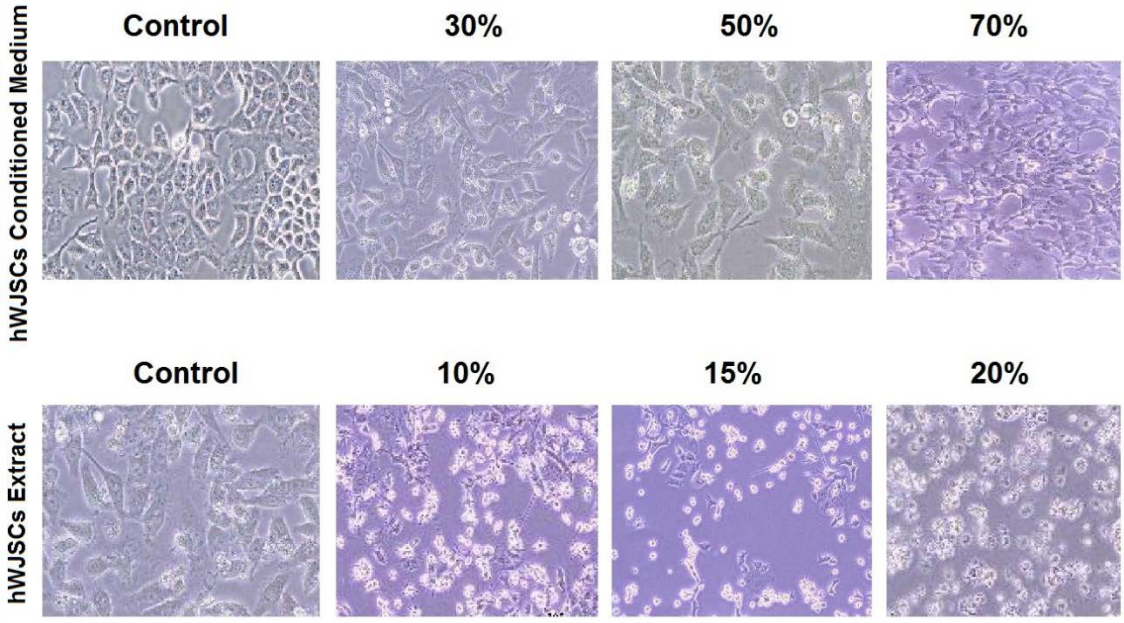

Figure 2. Cervical cancer cells' morphological alterations following 48 hours of treatment with the hWJSCs conditioned media and extract

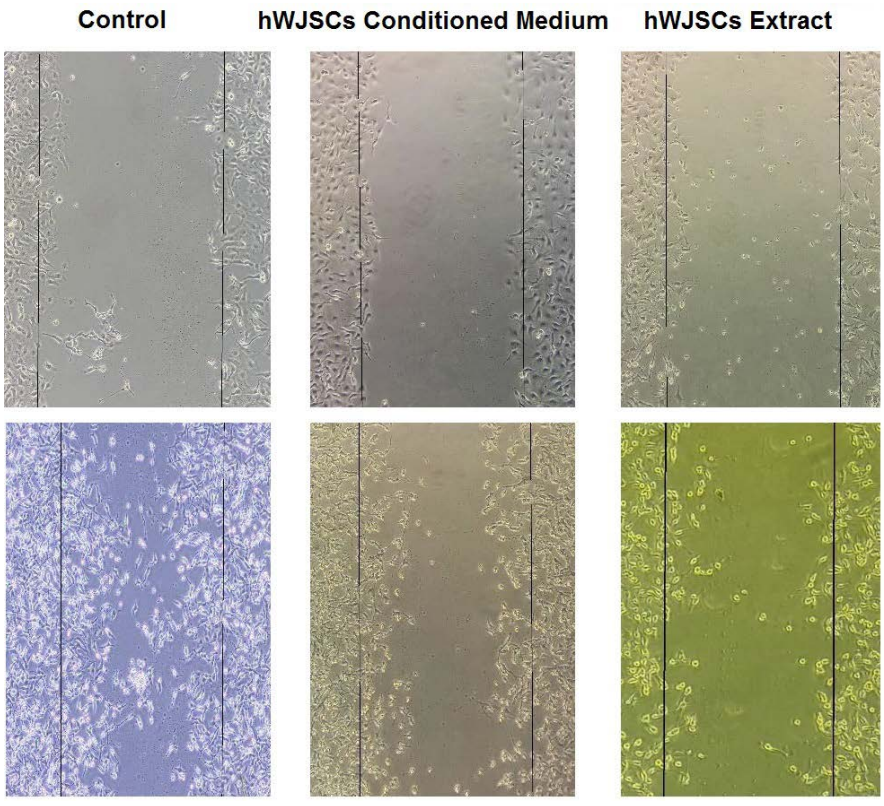

Figure 3. Cervical cancer cell migration after 72 hours of treatment with hWJSCs extract and conditioned media

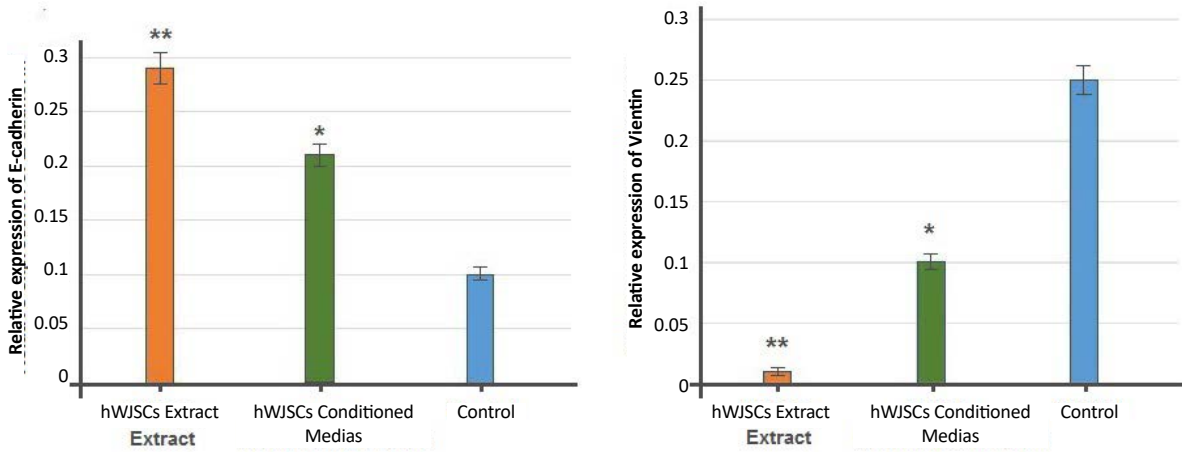

Figure 4. Expression of *E-cadherin* and *vimentin* genes in cervical cancer cells treated with hWJSCs extract and conditioned medias

phological modifications resulting in cell death. These changes included cell and nucleus shrinkage as well as cell membrane degradation. These morphological alterations are partly concentration-dependent (Figure 2).

Wound healing assessment with the migration assay

The effects of hWJSCs conditioned medium and hWJSCs extract on the migration of cervical cancer cells were evaluated using a wound healing test, which revealed that the conditioned media and extract significantly decreased cervical cancer cell migration (Figure 3).

Expression of *BAX* and *BCL-2* genes

The collected results showed that treatment of cervical cancer cells with the hWJSCs extract and conditioned media decreased *E-cadherin* gene expression by 2.1 and 2.9-fold, respectively. Contrarily, the conditioned media and hWJSCs extract increased the expression of the *vimentin* by 2.4 and 8 fold, respectively (Figure 4).

Discussion

E-cadherin and *vimentin* genes, which are involved in migration, as well as the Hela cervical cancer cell line, were examined in the current study to determine their effects on the conditioned medium and hWJSCs extract [16]. According to our research, the conditioned medium and hWJSCs extract hinder cervical cancer cells' ability to multiply and migrate in a concentration- and time-dependent manner. This could be a result of hWJSCs extraction and cell damage caused by conditioned media.

Various MSCs-derived conditioned media and extracts have already been studied on several cancer cells. Along with other paracrine substances released by the MSCs, vascular endothelial growth factor (VEGF) may have an impact on the viability and growth of cancer cells. The secretome of MSCs may affect tumor cells in this situation and inhibit their development [17]. The hWJSCs have been used as an anticancer drug in various earlier studies [18, 19]. These stem cells can be extracted painlessly and cheaply from the human umbilical cord, which was normally thrown away after delivery. The multipotency, robust proliferative capability, and long telomeres of hWJSCs make them display traits comparable to both embryonic and mesenchymal stem cells [20]. Unlike other mesenchymal stem cells, the hWJSCs do not cause tumor formation in mice with immune system suppression [21]. However, no research has been done on how these stem cells may affect cervical cancer.

We discovered that the conditioned media and cellular extraction limit cervical cancer cell proliferation and migration in a concentration- and time-dependent manner. In recent studies, cancer cells treated with MSCs showed a variety of morphological alterations that led to cell death [22, 23]. In ovarian cancer cells, the ablation of hWJSCs causes cellular damage, increased *caspase-3* gene expression, and modification of genes related to the cell cycle, which restricts cellular growth, proliferation, and death [24, 25]. Han et al. (2014) discovered that mesenchymal stem cells made from the human cord reduce the cellular development and migration of prostate cancer cells [26].

In the current study, the conditioned media and hWJSCs extract significantly increased the expression of the *E-cadherin* gene while significantly decreasing the expression of the *vimentin* gene in Hela cancer cells [27]. These genes are essential for the migration of cancer cells, which is a critical factor in metastasis [28, 29]. Cancer cells migrate more easily when *E-cadherin* levels are high, whereas cancer cells migrate less easily when *vimentin* levels are high [29, 30]. Controlling the expression of genes for *vimentin* and *E-cadherin* as well as other genes related to metastasis may therefore be a promising new treatment for cancer patients [31, 32]. Han et al. (2014) found that prostate cancer cells move less and experience death when MSCs derived from the human umbilical cord activate the JNK signaling pathway and inhibit the PI3K/AKT signaling pathway [26]. The expression of genes related to apoptosis was changed by the hWJSCs extract at a 50% concentration [21, 32]. To get accurate results, nevertheless, more studies with animal models and clinical investigations are required.

Conclusion

Overall, the results of this investigation showed that conditioned media and hWJSCs extract inhibit the migration of Hela cervical cancer cells by modifying the expression of genes related to migration. Nevertheless, more research is needed to pinpoint the precise ways that hWJSCs affect the physiopathology of cervical cancer.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by Tabriz Branch of Islamic Azad University (Code: IR.IAU.TABRIZ.REC.1398.028). All women signed a consent form and were informed about the study.

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Authors contribution's

The both authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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