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Human amniotic epithelial cells induce apoptosis of cancer cells: a new anti-tumor therapeutic strategy

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Abstract

Background aims. Amniotic membrane (AM), the innermost layer of human placenta, is composed of a single layer of epithelial cells, a basement membrane and an avascular stroma. The AM has many functions and properties, among which angiogenic modulatory and immunoregulatory effects are applicable in cancer therapy. Because these functions belong to amniotic epithelial cells, in this study we compared the anti-cancer effect of amniotic epithelial cells and the whole AM. *Methods.* The effect of the AM and the amniotic epithelial cells on cancer cell apoptosis was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium assay, terminal deoxynucleotidyl transferase dUTP nick end labeling assay and immunocytochemistry. The effect of the AM on angiogenesis in conditions both with and without epithelial cells was also evaluated using rat aortic ring assay. *Results.* There was a decrease in cancer cell viability after adding either AM or amniotic epithelial cell supernatant to cancer cells. A significant increase in caspase-3 and caspase-8 expression in cancer cells treated with amniotic epithelial cell supernatant was observed. The recorded media also demonstrated the possible induction of apoptosis in cancer cells treated with the amniotic epithelial cell supernatant. In the aorta ring assay, the AM showed an anti-angiogenic effect in the presence of its epithelial cells; however, this effect was altered to initiate angiogenesis when amniotic epithelial cells were removed from the AM. *Conclusions.* These results suggest that amniotic epithelial cells, with their anti-angiogenic effect and induction of apoptosis, are candidates for cancer therapeutic agents in the near future.

Key Words: *amniotic membrane, apoptosis, cancer, epithelial cells*

Introduction

Cancer is one of the leading causes of death worldwide, and despite continuous progress in management, it still imposes high mortality and morbidity on patients (1). Routine treatments for cancer such as surgery, chemotherapy and radiotherapy are not always effective and may be followed by recurrence or metastatic spread (2,3). Many new ideas have emerged more recently in the field of cancer treatment, many of which focus on the role of stem cells in cancer, such as targeting cancer stem cells (1,3), differentiation therapy, stem cell transplantation (4) and cancer gene therapy using neural stem cells in malignant glioma (5).

Among new ideas that deal with the role of stem cells in cancer treatment, application of amniotic membrane (AM) is a promising idea for cancer therapy. The AM, the innermost layer of placenta, is a biologic barrier that supports the fetus by preparing an anatomically, physiologically and immunologically privileged space. The AM is composed of a thick basement membrane with a single layer of epithelial cells and an avascular stroma containing mesenchymal cells (6). Amniotic epithelial and mesenchymal cells have stem cell characteristics, which make them a great source of stem cell therapy.

A key element in tumor growth and metastasis is the development of a proper blood supply for the newly formed tumor cells and destroying the body's immunologic barriers that prevent tumor invasion. The AM can prevent tumor metastasis because it has anti-angiogenic properties and immunoregulatory activity. Following the suggestion of these features for the AM, it was hypothesized that the AM can have anti-cancer effects (7,8). Other extra-embryonic cells, such as cord blood stem cells, also have been proven to have anti-cancer properties via a different mechanism, more likely apoptosis (9). The AM has also been shown to have an apoptotic effect on polymorphonuclear neutrophils (10) and

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macrophages (11) by secretion of certain substances. Apoptosis is a process of programmed cell death with significant roles in development and the homeostasis of multi-cellular organisms (12). Because the exact mechanism of the anti-tumor effect of the AM is unknown, the aim of this study was to evaluate whether the AM has anti-tumor properties through induction of apoptosis.

The origin of anti-cancer effect of the AM was the second question of this research. We hypothesized more recently that the amniotic epithelial cells are the source of anti-tumor properties of the AM (8). In this study, we evaluated the effect of the amniotic epithelial cells and the whole AM on proliferation of cancer cells.

Methods

The ethical committee of Shahid Beheshti University of Medical Sciences approved all experimental procedures. The placenta was obtained from mothers with normal pregnancy following elective cesarean section at gestational ages of about 36–38 weeks. Serologic tests for human immunodeficiency virus, hepatitis B and C and syphilis were negative for all cases, as checked before delivery. Although placenta is a waste material after delivery, adequate information about the study process and the usage of placenta was given to all parents who signed the informed consent and agreed to participate in this study.

Placenta tissues were transferred immediately to our laboratory, under sterile condition, at 4°C. The AM was separated from the chorionic membrane by a peeling method. After being washed thoroughly with phosphate-buffered saline (PBS) solution, the anti-cancer property of the AM was evaluated in different conditions.

The epithelial cells were harvested from the AM using enzymatic digestion by trypsin-ethylenediaminetetraacetic acid (EDTA) (TrypNik; Yavandan Andisheh, Tehran, Iran) 0.15%, as described previously (13). Briefly, to release amniotic epithelial cells, the AM was incubated at 37°C with 0.15% trypsin-EDTA. Cells from the first 10 min of digestion were discarded to exclude debris. The solutions from the second and third 40-min digests were pooled. Trypsin was inactivated with fetal bovine serum (FBS), and the solution was centrifuged at 2500 rpm for 12 min. Cells were washed with PBS and suspended in - Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA) containing 100 U/mL penicillin/streptomycin solution and 10% FBS. Viability of amniotic epithelial cells was determined by exclusion of trypan blue dye. The purity of the isolated cells was determined by immunostaining with antibody against the epithelial marker pan-cytokeratin conjugated with fluorescein isothiocyanate (1:100; Sigma-Aldrich).

Effects of AM supernatant on cancer cell viability

To evaluate the effects of the AM on viability and proliferation of cancer cells, the AM was used on two different cancer cell lines including HeLa cancer cells and breast cancer cells (MDA-MB-231) and in two different protocols, as follows: (i) The AM was cut into 12 small pieces that were each 2 × 2 cm, and the pieces were individually cultured epithelial side up in a 12-well plate. To each well, 1 mL of prepared DMEM containing FBS 10% and penicillin-streptomycin 1% was added. The plates were incubated in 37°C with 5% CO_2 for 24 h, after which the supernatant was collected from each well. (ii) The obtained epithelial cells were seeded on tissue culture collagen I (CollaNik I, Yavandan Andisheh, Tehran, Iran) coated plates at a density of 2×10^4 cells per cm^2 in DMEM containing FBS 10% and penicillin-streptomycin 1%, incubated at 37°C with 5% CO_2. After 24 h, the supernatant was separated from cells. In the amniotic epithelial cells group, the collected supernatant was filtered with 0.22-μm filters to remove all the possible epithelial cells from the medium.

The supernatant from both procedures was added to cancer cells, which were previously cultured in 24-well plates in prepared RPMI medium (Chemicon International, Temecula, CA, USA) (20% FBS and 1% penicillin-streptomycin) and incubated at 37°C with 5% CO_2, about 24 h before adding the supernatant. The amount of supernatant added to the cancer cells in each well ranged from 200–800 μL, added in increments of 200 μL. The cancer cells without treatment served as the control group. The cancer cells were incubated for another 24 h at 37°C. After 24 h, the vitality of the cancer cells, which had been cultured with the supernatant of the previously cultured amniotic epithelial cells or AM, was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium(MTT) assay.

MTT assay

MTT solution (5 mg of MTT/mL of distilled water) was filter sterilized. Solution was added to growing culture of cancer cells (40 μL in each well). The mixture was incubated for 4 h at 37°C. The cells were checked afterward, and the presence of crystal formation was evaluated. When the amounts of formed crystals among cells were acceptable, the formazan crystals were dissolved adding dimethyl sulfoxide (Sigma-Aldrich), 900 μL in each well. Formazan has maximum absorption in 570 nm.