

Anticancer Properties of Amniotic Membrane Epithelial Cells

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Abstract

Cancer is one of the important causes of mortality worldwide. Abundance of unsuccessful therapeutic methods, their side effects and the high cost of treatments, have led scientific societies to search novel targeting treatments. Anticancer properties of amniotic epithelial cells were emphasized previously, so in this study will review the anticancer factors secreted from this cell. These releasing factors including TNF- α , TNF- β , TGF- β , GM-CSF, NTF3, CCL18, BDNF, GCP-2, IL 2, 4, 6, and 8 induced apoptosis process in a variety of cancer cells. In sum up, this kind of stem cell with anticancer molecule are appropriated to use directly in cell therapy of cancer or indirectly use condition medium.

Introduction

After cardiovascular disease, cancer is the second mortality reason in USA, so that 1 out of 4 people die from cancer. Cancer prevalence has been reported as 454.8 cases between 2008 to 2012, and mortality rate as 171.2 out of 100 thousand people during the same years (16). Therapeutic costs for this disease in 2010 have been estimated about 125 milliard dollars in 2010 which will reach 156 milliards in 2020 (17). In spite of the ever-increasing advances in diagnostic methods and treatments for cancer, fatality rate is still high for this disease (1). Traditional therapeutic methods such as surgery, chemotherapy, and radiotherapy have not been that successful and despite these treatments, it is often characterized by relapse or metastasis because of cancerous cells spread by blood and lymph throughout the body, complexity of cancerous tissue, cellular heterogeneity, and presence of cancer stem cells. Furthermore, these methods have not been definitive and permanent cure and are accompanied by side effects due to their cytotoxic effects on normal cells (4). Besides, the increasing resistance of cancers to common treatments has become a problematic issue that leads

into therapeutic interventions failure. Therefore, considering all the mentioned problems and the two times increase in cancer occurrence during the recent decades, novel, efficient, and cost-effective therapeutic methods are necessary. Amongst all the under-discussion therapeutic ideas, cell-therapy, using natural human stem cells and genetically manipulated ones for gene therapy, differentiation therapy and transplant of these cells have gotten too much attention and extensive studies are being performed with therapeutic targets and even for eradicating different types of cancers. Fortunately, promising results have been obtained from recent studies (5-7). However, despite all the advances in clinical cell-therapy, this technology still encounters various problems including preparation of high-quality cells with suitable characteristics, lack of favorable effects, time consumption and high expenses for commercializing. Nowadays these challenges have been partially resolved through identifying the properties of amnion and its cells, and using these cells as new candidates for different diseases including cancer (8-17).

Cell therapy

Cell-therapy and stem cells usage are among novel therapeutic methods for different diseases which including cancer, about which extensive studies have been performed in recent years (18). Stem cells are capable self-renewal, and reproduce by cell division in the body. So they have become interesting during recent years for treating various diseases like cancer. These cells could be transformed into specialized cells with specific functions and activities in laboratory conditions. Stem cells are almost found in all the body tissues and are responsible for repair in fact. Based on their origin, these cells are categorized into two major groups of embryonic stem cells (ESCs) and adult stem cells (ASCs). ESCs are derived from epiblast tissue of inner cellular mass of blastocyst stage or the beginning of morula. These cells proliferate rapidly and spread in the culture medium. Since they are uniquely multipotent, they may differentiate into different adult tissue cells, and are theoretically a potent source for repair medicine and tissue replacement after injury or disease (19). Nevertheless, using these cells has lots of limitations these days. Several studies have described tumorigenesis of undifferentiated ESCs and teratoma formation after direct injection to the body (20). Additionally, the method for collecting these cells from human embryos might have remarkable ethical and moral issues. Therefore, treatment using ESCs has not yet received license (21).

Adult stem cells which are found both in children and adults are multipotent and have limited differentiation ability, so that they show their tissue origin including mesenchymal stem cells, fat tissue derived stem cells, endothelial stem cells, nervous stem cells, etc. Unlike ESCs, utilizing adult stem cells in researches or for therapy is not challenging. Besides, adult stem cells could be obtained from the intended receiver because they also match immunologically. Adult stem cells are extensively being used for research about different cell-based treatments. For example, the previous studies reported that transplanting multi potential human stem cells which are derived from bone marrow results in antitumor activity against non-hodgkin

lymphoma (22). Nonetheless, it has been shown that some of these cells cause increased growth of large intestine cancer, lymphoma, and melanoma cells (23, 24). Thus, it is not yet clarified whether these cells further or suppress tumor growth. Problems for isolating adult stem cells and dangers in collecting these cells from different tissues such as bone marrow, blood, fat tissue, and brain, and spreading them in laboratory conditions are other limitations for using these cells in therapeutic methods. Therefore, regarding the mentioned challenges, many studies have focused on searching for new stem cells to be effectively used for therapeutic purpose without any limitation (6). Identifying amnion-derived cells characteristics have made them a suitable source for stem cells-based treatments (25).

Amniotic Membrane Stem Cells

Amnion is a transparent membrane which lacks nerves, muscles, and lymphatic vessels, and provides a safe space for fetus growth and development along with chorionic membrane. Amniotic membrane thickness is between 20 to 200 micrometers and consists of three histological layers including epithelial, basal layer, and mesenchymal tissue. Human amniotic epithelial cells (hAECs) are the main cell population of amnion and are in direct contact with amniotic fluid. These cells have been described with cubic morphology, large atypical nucleus, intracytoplasmic organelles and pinocytotic vesicles, and are homogeneously distributed on basal layer and form the inner layer of amnion (10). Amniotic epithelial cells have unique characteristics such as similarity to fetal stem cells, immune regulatory features, anti-fibrosis, anti-inflammation, anti-angiogenesis, meanwhile not being tumorigenic (11). In addition, these cells show low to intermediate expression of antigens like HLA-A, HLA-B, HLA-C and low expression of HLA-D and HLA-QR and lack of HLA-G expression, and consequently they do not provoke immunologic reactions and graft rejection after transplantation to human and animal models due to low immunogenicity. Besides, this cellular source is easily available and using it does not have ethical issues like fetal stem cells

(12-14). Therefore, identifying these properties during recent years has introduced these cells as a suitable candidate for treating cancer. Based on the performed studies, researchers have stated that some factors in the supernatant of amnion epithelial cells culture result in cell cycle arrest and apoptosis induction in cancerous cell line (15). However, the type of existing effective material and its effect mechanism have not been clearly determined yet. Thus, considering the cancer treatment problem, little literature about the anticancer potential of factors derived from amnion epithelial cells, and the unknown nature of these factors, so more studies for identifying these factors seems to be necessary.

One of the unique properties of these cells is low expression of major histocompatibility complex (MHC). Consequently, they could reduce graft rejection possibility in allogenic transplants due to low immunogenicity (26, 27). These cells also have less limited differentiation potential and regarding this potency they are between multipotent ESCs and adult stem cells with limited lineage.

One of the main concerns for applying stem cells for treatment is their tumorigenic property which limits using ESCs despite their high clinical potency. Although they are capable of active self-renewal and widely spread, tumorigenesis and teratoma formation in the body has not been recorded for stem cells derived from amnion (28). Tumorigenesis has not been observed after transplanting these cells to human volunteers which is beneficial for clinical usage of these cells (29). On the other hand, spontaneous chromosome changes were not seen in consecutive passage of these cells and their chromosomal stability has been proved by karyotype analyses (30). Additionally, there is no ethical issue in stem amnion-derived cells applying programs and their origins such as amnion membrane and its fluid, are easily available.

Thus, using this cellular population as a suitable candidate for cell-therapy has attracted much attention (31).

Amnion is the membranous sac that surrounds fetus and amniotic fluid and is

responsible for protecting the embryo during growth and development stages. This sac also plays an important role during delivery through inducing prostaglandins biosynthesis, which are essential for initiation and continuing of uterus contractions (29).

Amniotic Epithelial Cells

Amnion arises from growth of tissues out of embryo. It consists of three main layers, one of which being human amnion epithelial cells (hAECs), one basal layer or thick basal membrane and a mesenchymal layer which lacks blood vessels. Amnion is a tissue which lacks any kind of blood vessel, nerve, muscle or lymphatic structures, and nutritious material and other needed material are obtained directly through diffusion mechanism from amniotic fluid or from the inner layer, that is the placenta and its components. Amniotic epithelium is a layer consisting of uniform cells on basal membrane and in direct contact with amniotic fluid. Amniotic epithelial stem cells are derived from this layer. There is a condensed layer under the basal membrane which lacks cells and is the main fiber skeleton of amnion membrane and includes collagen clusters secreted by mesenchymal cells present in fibroblast layer. Stromal fibroblast is the thickest amniotic cellular layer that contains fibroblast-like mesenchymal cells, and amniotic mesenchymal stem cells are derived from that (28).

Human amnion has special characteristics, among which inhibiting bacterial growth, preventing inflammatory reactions in immune system, preventing wound development, helping wound repair, and accelerating epithelialization could be mentioned. These properties have caused wide usage of amnion in medicine (9, 20). Studies on amnion and its clinical application go back to 1910, when the thin and almost transparent tissue of amnion was used for the first time by Davis as surgical material for skin repair (32). Short time after that, its application for treating skin and burn got extended. In 1940, successful use of amnion in conjunctival infections was reported (33). Various reports were then provided regarding human amnion transplant for corneal and

conjunctival renewal (34-36). Nowadays, in addition to ophthalmologic applications, amnion has wide usage in surgery and treatments for skin burn, bed wound, ulcers and artificial vagina repair (37). In 1981, Aike et al. transplanted amnion to some human volunteers in order to evaluate its immunogenicity, and no sign of infective disease, immunologic reaction and inflammation was seen in examinations. In 1982, Adinolfi et al. reported lack of HLA-A-B-C-DR antigens and beta-2 macroglobulin expression, and lysosomal enzymes expression by human amnion epithelial cells, and this cell population has been proposed as a candidate for treating genetic diseases of lysosomal enzymes deficiency (38).

Pluripotential properties of hAECs were first stated by Tamagawa et al. in 2004. They produced chimera xenogeneic mouse in vitro using amnion cells and mouse embryo cells and showed differentiation of these cells into three embryonic layers through forming embryo body. In addition, the results of their study were indicative of hAEC cells normal karyotype used after 60 passages (39). Other studies demonstrated characteristics such as the similarity between hAECs superficial markers and stem cells, colony forming ability, and differentiation into three embryonic layers and lack of tumorigenesis for these cells (12, 40, 41).

hAECs express superficial protein markers of embryonic stem cells such as SSEA-3, SSEA-4, TRA-1-60, TRA-1-80, Thy-1, C-Kit (20, 43). Furthermore, these cells express other factors related to stem cells pluripotency and self-renewal including Oct3/4, SOX2, FGF-4, Rex-1, BST-1, ABCG2, and NANOG, and normal stem cell markers such as nestin, vimentin, and neuron-specific enolase (12, 44). Molecules involved in neuronal development including acetylcholine and catecholamines are also produced and secreted by these cells. Besides, neurotrophic factors like neuronal growth factor, neurotrophic factor derived from brain and neurotrophin-3 have been identified in hAECs and amniotic fluid (45). More researches on amnion epithelial cells have shown that these cells are pluripotent and are capable of differentiation into all the three mesoderm, ectoderm, and endoderm

layers (9). Moreover, hAECs are immunologically neutral and this phenomenon decreases the risk for graft rejection or immunological reactions after transplanting these cells. These cells do not express HLA-A, -B, -C or -DR antigens, so that they are less probable to be rejected by host immune system and transplanting these cells is easier (11, 38). The supernatant of these cells inhibits innate and acquired immune system through secreting soluble factors such as MIP-2, FasL, TRAIL, TGF β , and MIF (46).

Using novel treatments including cell-therapy and tissue engineering in various lesions repair and different diseases treatment has been ever-increasing. On the other hand, there is potency for employing amnion epithelial cells and the amnion itself in these treatments (11, 38). For example, Maronigue et al. reported that hAECs could be used as hepatocyte source derived from stem cell in transplantation methods for treating liver diseases. hAECs in this study differentiated into hepatocyte-like cells after transplant into mouse liver. Therefore, hAECs are capable of differentiation into cells with hepatocytes functions both in vitro and in vivo (47). There are also extensive documents regarding hAECs differentiation to neurons, cardiomyocytes, myocytes, osteocytes, and adipocytes (15, 48, 49).

In addition to all these features, amnion and its epithelial cells are not tumorigenic, and do not have the most important limitation of treatment by ESCs and iPSCs. In the performed studies about injection and implant of amnion and its cells in different human and animal organs, did not stimulate the host immune system and any sign of tumorigenesis has not been observed (50).

Anticancer Properties of Amniotic Epithelial Cells

Anticancer properties theory for amnion was first presented by Seo et al. in 2008. They proposed amnion and epithelial cells for treating cancer on the strength of anti-angiogenesis activities, immunoregulatory effects, and pro-apoptotic properties of these cells (11). Afterwards, some studies were done in order to evaluate this hypothesis by research teams of Kim, Kang, Niknejad, and

Mamede (10, 41, and 42). Further studies on several cancerous cell lines confirm the antitumor property of amnion cells (51). The antitumor characteristics of these cells is due to releasing fatal factors and cytokines for tumor cells such as TNF- α , TNF- β , transforming growth factor- β , macrophage-granulocyte colony stimulating factor, neurotrophin-3, CCL18 (chemokine), macrophage stimulating factor, brain-derived neurotrophic factor, granulocytic chemotactic protein (GCP-2), protected dopamine neurotrophic factor, and interleukins 2, 4, 6, and 8 (9, 21, 52). It also seems as if there are unknown factors which result in these antitumor properties of amnion. Cancerous cells lead into formation of new vessels from present vessels and induce angiogenesis through producing and secreting special mediators. The molecular mechanism for the anticancer effect of the fluid resultant from human amnion epithelial cells is not well known, but the studies show that this fluid contains a soluble anticancer material which inhibits angiogenesis and could be utilized for preventing growth and metastasis of tumors (11, 53, 54). Different reports have assigned the anti-angiogenesis properties of these cells to the anti-angiogenesis factors secreted by epithelial cells like trombospondin-1, IL-1 receptor antagonists, IL-10, and some tissue inhibitor metalloprotease's (TIMP-1, TIMP-2, TIMP-3, TIMP-4) (11, 53, 55).

In the study of Kang et al. in 2012 the strategy for human stem cells transplant as a therapeutic method for treating different types of cancer including breast cancer was evaluated. Natural hAECs which had not been engineered were used as cell source for human stem cells transplantation. The results showed that co-culture of hACEs and breast cancer cells of MDA-MB-231 with the ratio of 1:4 or 1:8 (tumor cells to stem cells), inhibits growth of breast cancer cells 67.29 and 67.33, respectively, while it does not affect bovine fibroblast cells as control group. Moreover, these cells demonstrated remarkable antitumor effects without any side effects (such as weight loss, mortality, and bruise) compared to mouse that just received 5-FU treatment, when were injected to mouse with tumor of these

cancerous cells (ratio of 1:4). Besides, breast tissue showed favorable survival in treatment by hACEs in this study (9).

Niknejad et al. (2014) attributed the amnion anticancer properties hypothesis to secreting factor of amnion epithelial cells. While examining the effect of secretory fluid obtained from epithelial cells on cervix cancer cells (Hela cell line) and breast cancer (MDA-MB-231), they found that this fluid causes reduction in viability and proliferation of these cells, and this procedure is dose-dependent, so that proliferation and viability of cancerous cells decreases by increasing the concentration of condition medium for amnion and epithelial cells (15). Morphological and molecular studies also show cell death mechanism through apoptosis.

As has previously been reported about cell death mechanism due to amniotic fluid, death occurs as a result of cell cycle arrest in G0/G1 stage of the cell cycle. Function mechanism for the fluid secreted from epithelial cells also shows cell cycle arrest in this stage. Elevated expression of caspase 3 and 8 in cancerous cells under the effect of secretory fluid from epithelial cells has been observed (15). Niknejad et al. stated in another study that unknown materials in the culture medium resulting from amnion epithelial cells downregulated HSP90 and the related proteins, thereby led into cell cycle arrest, apoptosis induction, and angiogenesis inhibition due to HSP90 regulatory role in these procedures (56).

Mamede (2014) evaluated the effects of proteins secreted from amnion on metabolic activity of different cancerous cell lines regarding the anticancer effects of human amnion. Results of this study confirm the difference between effects of these proteins on different cell lines of a cancer type, and Mamede related these impacts to the genetic profile of these cell lines (51). The only present study that has evaluated the nature of existing materials in amniotic fluid, indicated that among the four studied fractions, just the fraction containing components with molecular weight of less than 3 kilodaltons had inhibitory effect against lymphocytes proliferation.

Additionally, mixture of the two fractions of less than 3 and more than 100 kilodaltons demonstrated more inhibitory impact. The results obtained from this study confirmed that soluble factors which were responsible for the anti-proliferative effect on lymphocytes had low molecular weight (under 3KDa), were heat-resistant, and likely non-protein. Prostaglandin was identified as one of the key molecules of this procedure in this study (57).

Conclusion

Hence, considering the probability of different soluble factors presence in the fluid resultant from amniotic epithelial cells

culture and their different action mechanisms on cancerous cells, performing similar studies on cancerous cells for identifying the exact nature of these factors and clarifying their activity seem to be necessary. The mentioned aims could be achieved through performing proteomics, transcriptomics, metabolomics, and peptidomics studies on amnion epithelial cells-derived culture medium.

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