Cancer Gene Therapy (2012) 19, 517 – 522

www.nature.com/cgt

REVIEW

Potential antitumor therapeutic strategies of human amniotic membrane and amniotic fluid-derived stem cells

N-H Kang[1], K-A Hwang[1], SU Kim[2,3], Y-B Kim[1], S-H Hyun[1], E-B Jeung[1] and K-C Choi[1]

As stem cells are capable of self-renewal and can generate differentiated progenies for organ development, they are considered as potential source for regenerative medicine and tissue replacement after injury or disease. Along with this capacity, stem cells have the therapeutic potential for treating human diseases including cancers. According to the origins, stem cells are broadly classified into two types: embryonic stem cells (ESCs) and adult stem cells. In terms of differentiation potential, ESCs are pluripotent and adult stem cells are multipotent. Amnion, which is a membranous sac that contains the fetus and amniotic fluid and functions in protecting the developing embryo during gestation, is another stem cell source. Amnion-derived stem cells are classified as human amniotic membrane-derived epithelial stem cells, human amniotic membrane-derived mesenchymal stem cells and human amniotic fluid-derived stem cells. They are in an intermediate stage between pluripotent ESCs and lineage-restricted adult stem cells, non-tumorigenic, and contribute to low immunogenicity and anti-inflammation. Furthermore, they are easily available and do not cause any controversial issues in their recovery and applications. Not only are amnion-derived stem cells applicable in regenerative medicine, they have anticancer capacity. In non-engineered stem cells transplantation strategies, amnion-derived stem cells effectively target the tumor and suppressed the tumor growth by expressing cytotoxic cytokines. Additionally, they also have a potential as novel delivery vehicles transferring therapeutic genes to the cancer formation sites in gene-directed enzyme/prodrug combination therapy. Owing to their own advantageous properties, amnion-derived stem cells are emerging as a new candidate in anticancer therapy.

Cancer Gene Therapy (2012) **19,** 517–522; doi:10.1038/cgt.2012.30; published online 1 June 2012

Keywords: amniotic membrane; fluid-derived stem cells; tumor; transplantation; suicide gene

INTRODUCTION

Stem cells have recently received a great deal of attention because of their therapeutic potential for treating human diseases including cancer.[1–3] Stem cells are capable of self-renewal and can generate differentiated progenies for organ development. The differentiation potential is specified as cell potency and stem cells have various degrees of potency such as totipotency, pluripotency and multipotency.[4] According to the origin of stem cells, they are broadly classified into two types: embryonic stem cells (ESCs) isolated from the inner cell mass of blastocysts, and adult stem cells found in various tissues.

Human ESCs (hESCs) are derived from the epiblast tissue of the inner cell mass of a blastocyst or earlier morula stage of embryo.[5] They can quickly proliferate, unlimitedly expand in culture and differentiate into diverse cell types of all adult tissues because of their unique pluripotency.[6] Therefore, hESCs have been considered as a theoretically potential source for regenerative medicine and tissue replacement after injury or disease. However, they are currently facing major limitations for use. Some investigations have described the tumorigenicity of undifferentiated hESCs[7] and the possibility of teratoma formation when injected directly into other bodies.[8] Moreover, the procedures used to collect hESCs from human embryos are associated with substantial moral and ethical issues.[9] Therefore, the treatments using ESCs are not approved at present. Adult stem cells are also known as somatic or germline stem cells and are found in children as well as adults.[10] Most adult stem cells are multipotent, lineage-restricted and are generally referred to by their tissue origins: mesenchymal stem cell (MSC), adipose-derived stem cell, endothelial stem cell and neural stem cell, and so on.[11] Unlike hESCs, the use of adult stem cells in research or therapy is not controversial. Additionally, as adult stem cells can be obtained from the intended recipient for therapeutic purpose, they are immunologically compatible in this case. Adult stem cells have been widely used for research on various cell-based therapies. For instance, previous study reported that bone marrow-derived human multipotent MSCs transplanted resulted in antitumor activity against non-Hodgkin's lymphoma.[12] However, some MSCs have been also shown to increase the *in vivo* growth of colon cancer, lymphoma and melanoma cells.[13,14] Therefore, it is unclear whether MSCs promote or suppress tumor growth so far. The difficulties of isolating adult stem cells from diverse tissues such as bone marrow, blood, adipose tissue and brain and expanding them *in vitro* are another limiting points in their applications because harvesting procedures involve the risks and usually a small number of stem cells can be obtained in a single procedure.[15] Therefore, many studies have been focusing on searching for novel stem cells that can be effectively used for therapeutic purposes without any restrictions.

Recently, amniotic-derived stem cells isolated from human amnion and amniotic fluid have been considered as another potential candidate for stem cell-based therapies and they include human amniotic membrane-derived stem (hAMS) cells and

[1]Laboratory of Veterinary Biochemistry and Immunology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk, Republic of Korea; [2]Division of Neurology, Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada and [3]Medical Research Institute, Chung-Ang University College of Medicine, Seoul, Republic of Korea. Correspondence: Dr K-C Choi, Laboratory of Veterinary Biochemistry and Immunology, College of Veterinary Medicine, Chungbuk National University, Gaesin-dong 12, Cheongju, Chungbuk 361-763, Republic of Korea.
E-mail: kchoi@cbu.ac.kr
Received 19 September 2011; revised 23 April 2012; accepted 26 April 2012; published online 1 June 2012

amniotic fluid-derived stem (hAFS) cells.[16–18] They are known for having unique characteristics such as the low expression level of major histocompatibility complex antigens, which may contribute to causing low immunogenicity and anti-inflammation when introduced to other bodies, and a less restricted differentiation potential. In terms of the differentiation potential, they are in an intermediate stage between pluripotent ESCs and lineage-restricted adult stem cells, expressing the transcription factor Oct4 that has a critical role in maintaining pluripotency, but not accomplishing whole differentiation into the three embryonic germ layers (ectoderm, mesoderm and endoderm) and teratoma formation *in vivo*.[8,16,19] Hence, they are often referred to multipotent stem cells and can differentiate into diverse cell types such as adipogenic, osteogenic, myogenic, endothelial, hepatic and also neuronal lines.[17] These stem cells are able to actively self-renew, expand extensively and are not tumorigenic.[8] Additionally, as the applications of amniotic-derived stem cells do not have any ethical objection and their original sources including amniotic membrane and fluid are easily available, they have earned much attention. In this review, we will discuss the possible role of amniotic-derived stem cells for various antitumor modalities as new candidates of human stem cells.

CLASSIFICATION OF AMNIOTIC MEMBRANE/FLUID-DERIVED STEM CELLS

The amnion is a membranous sac that contains the fetus and amniotic fluid, and functions in protecting the developing embryo against various stimuli from the surroundings.[17] It also has an important role during parturition by enhancing the biosynthesis of prostaglandins, which are necessary for the initiation and maintenance of uterine contraction.[15] The amniotic membrane is composed of three major layers of a single epithelial layer, a basement membrane and an avascular mesenchyme (Figure 1). Although none of the three layers contain any nerves, muscles, blood vessels or lymphatic structures, the required nutrients are directly supplied to fetus by diffusion from the amniotic fluid and underlining deciduas.[8]

The amniotic epithelium is a monolayer composed of cells uniformly arranged on a basement membrane and in direct contact with the amniotic fluid. The cells of amniotic epithelium secrete glycoproteins, collagens and laminins that comprise the basement membrane, which is supporting the fetus.[8,20] The amniotic epithelial stem cells are derived from this layer. Under the basement membrane, there is an acelluar compact layer that is the main fibrous skeleton of the amniotic membrane and composed of collagen bundles secreted by mesenchymal cells present in the fibroblast layer. The stromal fibroblast layer is the thickest amniotic cell layer and contains fibroblast-like mesenchymal cells, from which amniotic membrane MSCs are derived.[21] Amniotic fluid filling the inner space of amnion is also an important source of the diverse cell types derived from the developing embryo as well as the amniotic membrane.[8,16,17] Therefore, various types of stem cells such as amniotic membrane-derived epithelial, amniotic membrane-derived mesenchymal and amniotic fluid stem cells can be obtained from the amniotic fluid.

STEM CELL CHARACTERISTICS OF AMNIOTIC MEMBRANE/FLUID-DERIVED STEM CELLS

Human amniotic membrane-derived epithelial stem cells (hAECs)

hAECs are originated from the epithelial layer of amniotic membrane and easily isolated from amnion or amniotic fluid that is readily available during gestation and at the time of birth. These cells have been shown to express the stem cell surface marker proteins such as SSEA-3, SSEA-4, TRA 1-60, TRA 1-81, Thy-1 and c-kit. Besides these stem cell markers, hAECs express other molecular markers including OCT4, SOX2 and NANOG, which are required for self-renewal and/or pluripotency.[8,19,22–27] Therefore, hAECs are shown to be able to trilineage differentiation *in vitro*

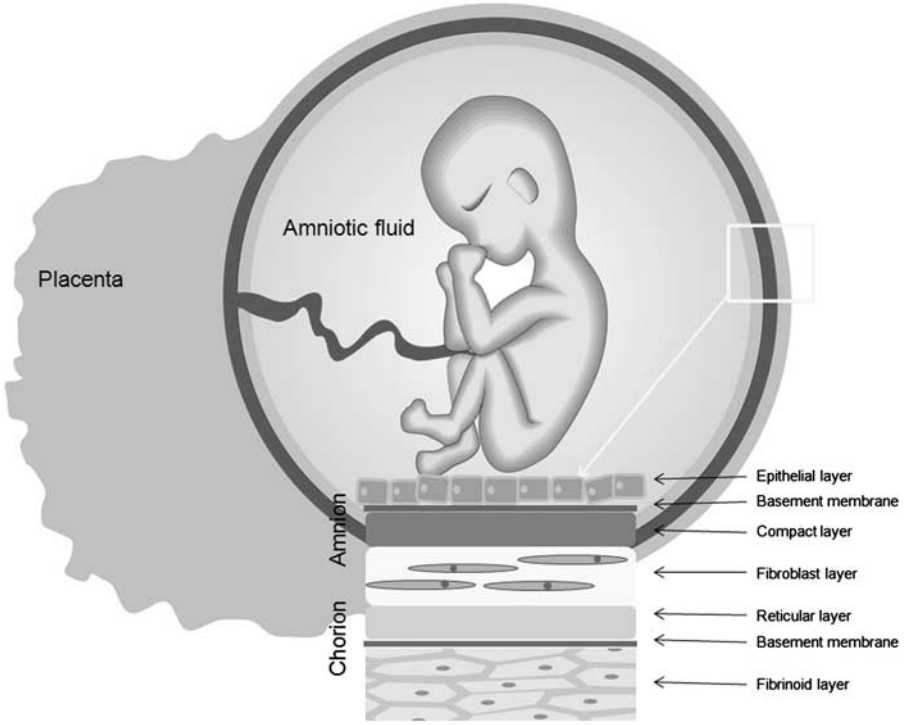

Figure 1. The amniotic membrane and amniotic fluid composition. The amniotic membrane is composed of three major layers: a single epithelial layer, a thick basement membrane and an avascular mesenchyme. Amniotic fluid contains diverse types of cells from all three germ layer derived from the skin and digestive tract of the developing embryo, and from the amniotic membrane.

such as differentiations into cells of the pancreatic and hepatic endoderm, neuro-ectoderm and cardiac mesoderm lineages.[28] This differentiation ability of hAECs toward diverse cell types has been actually studied to apply for the therapeutic purposes. For example, Marongiu *et al*.[29] reported that hAECs may serve as a source of stem cell-derived hepatocytes in transplantation procedures for treating liver disease. After transplantation into the livers of retrorsine-treated SCID/beige mice, naive hAECs differentiated into hepatocyte-like cells. Thus, hAECs possess the ability to differentiate into cells with characteristics of functional hepatocytes both *in vitro* and *in vivo*. Furthermore, more extensive studies documented the various differentiations of hAECs into neuron, astrocytes, pancreatic cells, cardiomyocytes, myocytes, osteocytes and adipocytes.[30]

Human amniotic membrane-derived MSCs (AM-hMSCs)

Amniotic membrane-human MSCs are isolated from amniotic membrane[8,16,24,26] and named AM-hMSCs. AM-hMSCs can be obtained after *in vitro* culture of term amnion. As the same sort of mesenchymal cells, they have a lot in common in immunophenotypical profile with human bone marrow-derived mesenchymal stromal cells.[31] However, AM-hMSCs express higher levels of *OCT4* compared with bone marrow-derived mesenchymal stromal cells, thereby being superior to adult MSCs in their proliferation and differentiation potential.[31] In addition, they are positive for *SOX2, REX1, NESTIN* (neural stem cell marker), *NANOG* and *CF* (stem cell factor, ligand of c-kit).[21,32] In terms of differentiation potential, AM-hMSCs were shown to differentiate in culture toward different cell types such as adipogenic, chondrogenic, osteogenic, skeletal- and cardio-myogenic, endothelial cells, hepatocyte-like cells and neuroglial cells.[33] Zhang *et al*.[26] have reported that the transplantation of AM-hMSCs significantly decreased hepatic fibrosis and progression of CCl_4-induced cirrhosis, thereby providing a new approach for the treatment of fibrotic liver disease. In addition, when transplanted in rat heart, they were also shown to survive and to achieve cardiac differentiation without immunological rejection.[34]

Human amniotic fluid-derived stem cells (hAFCs)

As human amniotic fluid contains the developing embryo and directly contacts with the amniotic membrane during the gestation period, it has diverse cell types derived from an embryo and the amniotic membrane.[17,35] The presence of hAFCs population in amniotic fluid was firstly identified in 2002 by Prusa *et al*.[36] and since then, their stem cell characteristics have been extensively studied. hAFCs was shown to express *OCT4* mRNA[37] and was scored positive for mesenchymal markers such as CD90, CD105 (SH2), CD73 (SH3/4) and CD166, but negative for hematopoietic markers.[33] Therefore, hAFCs have similar characteristics to human bone marrow MSCs, but are less differentiated.[15,38] Being *OCT4* positive, hAFCs are capable of extensive self-renewal and can differentiate into cells types of the three germ layers such as adipogenic, osteogenic, myogenic, endothelial, neurogenic and hepatic cells.[8,16,32]

NON-TUMORIGENICITY OF AMNIOTIC MEMBRANE/FLUID-DERIVED STEM CELLS

Tumorigenicity can be a main concern in using stem cells as therapeutic purposes for they have namely the ability to self-renew and proliferate. Among various types of stem cells, the tumorigenicity is closely linked with the pluripotent stem cells like ESCs. Pre-clinical studies in animal models have reported teratoma formation occurring on injection of mouse ESCs, hESCs or ESC-derived cells.[1–4] Therefore, hESCs have a major hurdle to overcome before clinical translation despite their great potential for regenerative medicine along with the ethical concerns. On the other hand, *in vivo* teratoma formation and tumorigenicity of human amniotic-derived stem cells have not been reported.[15] Actually, when they were transplanted into human volunteers, the non-tumorigenicity has been observed.[15] De Coppi *et al*.[17] also reported that hAFS cells, unlike ES cells, do not form tumors *in vivo* and their low risk of tumorigenicity would be advantageous for eventual therapeutic applications. For the details, Sessarego *et al*. isolated hAM-MSCs and demonstrated their non-tumorigenicity by karyotype analysis. The karyotype of hAM-MSCs was analyzed at different culture passages (fourth and sixth) to monitor the occurrence of spontaneous chromosomal alterations during expansion. The results of this study indicated that hAM-MSCs do not have tumorigenic potential.[12] Phermthai *et al*.[35] also demonstrated their chromosomal stability by karyotype analysis. When a clonal hAFS cell line was examined after prolonged *in vitro* culture, hAFS cells appeared to lack chromosomal mutations associated with tumorigenicity because of their chromosomal stability. Luo *et al*.[25] did not detect any tumor formation when hAECs expressing *AAV-GFP* (a green fluorescent protein gene) were transplanted into the spleens of SCID nude mice as a partial hepatectomy model. Therefore, it has been concluded that hAMS and hAFS cells may be potentially useful cell source given their non-tumorigenicity, and may be used for various stem cell-based therapeutic procedures.

ANTITUMOR THERAPEUTIC STRATEGIES WITH AMNIOTIC MEMBRANE/FLUID-DERIVED STEM CELLS

Cancer therapeutic strategies include surgery, radiotherapy and chemotherapy. Despite the developments in therapy, cancer mortality rates are high worldwide. Although useful, these modalities do not offer a permanent cure and rather cause various side effects.[39–44] For example, conventional chemotherapeutic drugs, including 5-fluorouracil, are widely used but are associated with various side effects including myelo-suppression, nausea, vomiting, diarrhea and stomatitis. Consequently, there are substantial needs for novel, low toxicity and effective anticancer therapies.[45,46] Current potential anticancer strategies using stem cells have been regarded as a novel candidate in medical fields and they involve the protocols conducted by either non-engineered or engineered stem cells.

Non-engineered stem cells transplantation strategies

Recently, human stem cell transplantation therapy has drawn attention as an upcoming alternative therapeutic tool in regenerative medicine as well as for the treatment of various diseases including cancer. Several studies have suggested that various human stem cells display antitumor effects.[9,12,23,35,47–51] For example, a study by Ayuzawa *et al*.[9] demonstrated the significant antitumor effect of transplanted human stem cells. Naive human umbilical cord matrix-derived stem cells were found to significantly attenuate the growth of human breast cancer cells *in vitro* and *in vivo*. When co-cultured with MDA 231 human breast carcinoma cells, human umbilical cord matrix-derived stem cell significantly inhibited DNA synthesis in the MDA 231 cells in a dose-dependent manner, thereby leading to tumor growth suppression. Ayuzawa *et al*.[9] also claimed that human umbilical cord matrix-derived stem cell produced factors such as nitric oxide and various cytokines that attenuate cancer cell growth, perhaps by altering the cell cycle. Furthermore, human fetal skin-derived MSCs inhibited the growth of MCF-7 breast cancer cells *in vitro*.[52] Cho *et al*.[53] reported that hyperthermia-treated MSCs (heat-treated MSCs) exerted antitumor effects on human carcinoma cells by producing various cytokines such as interleukin (IL) (Table 1).

Human amniotic membrane/fluid-derived stem cells may have therapeutic potential *in vitro* and *in vivo* and derived promising results as mentioned above. hAMS or hAFS are presumed to

Table 1. The list of previous studies reporting therapeutic potential of human amniotic membrane/fluid-derived stem cells

Stem cell	*Disease (animal model)*	*Reference*
hAECs	CCl_4-induced liver fibrosis	Manuelpillai *et al*.[55]
hAFCs	Acute kidney injury	Hauser *et al*.[77]
hAECs	Retrorsine-induced liver disease	Marongiu *et al*.[29]
hAM-MSCs	Glioma	Jiao *et al*.[24]
hAECs	Partial hepatectomy	Luo *et al*.[25]
hAM-MSCs	CCl_4-induced cirrhosis	Zhang *et al*.[26]
hAECs	Breast cancer	Kang *et al*.[54]

Abbreviations: hAEC, human amniotic membrane-derived epithelial stem cell; hAFC, human amniotic fluid-derived stem cell; hAM-MSC, human amniotic membrane-derived mesenchymal stem cell.

express various cytokine factors. When hAMS or hAFS cells are co-cultured with human carcinoma cells, the cancer cell viability can be decreased by the presence of hAMS or hAFS expressing cytotoxic factors, that is, tumor necrosis factor-α, transforming growth factor-β, ILs or interferons. In the previous study, we also detected the expressions of tumorcidal factors or cytokines in amniotic-derived stem cells. In a cytokine array analysis and reverse transcription-PCR, hAECs were demonstrated to secrete the various cytokines such as tumor necrosis factor-α, tumor necrosis factor-β, transforming growth factor-β, interferon-γ, IL-2, IL-3, IL-4, macrophage colony-stimulating factor and IL-8. When co-cultured with MDA-MB-231 breast cancer cells *in vitro*, hAECs decreased the viability of cancer cells. In the mouse models xenografted with MDA-MB-231 cells, hAECs effectively targeted the breast tumor and suppressed the tumor volume.[54] Therefore, hAECs appear to serve as mediators that inhibit tumor growth by expressing various cytokines that facilitate tumor cell death. Recently, many other reports also support the relationship between the expression of cytokines in hAMS or hAFS and antitumor effect. It is said that the tumor suppression effect was due to various cytokines produced by AM-hMSCs such as granulocyte macrophage colony-stimulating factor, IL-6, neurotrophin 3, CCL18 (a chemokine), macrophage colony-stimulating factor, brain-derived neutrophic factor, granulocyte chemotactic protein (GCP-2) and conserved dopomine neutrophic factors.[53] In detail, AM-hMSCs may mediate the tumor-targeting effects of immunocytes by regulating the expressions of various cytokines such as tumor necrosis factor-α and ILs.[55] For instance, ILs may help immune systems by activating natural killer cells that can directly target human cancers. The results from these studies demonstrated that hAMS and hAFS have potential antitumor effects.

Engineered stem cells strategies

Gene therapies using stem cells are widely applied for treating diseases including cancer.[39,44,56–60] Suicide genes can be effectively used for these procedures.[43,61,62] As suicide gene can cause a cell to kill itself and surrounding cells via bystander effects,[63–66] it can selectively lead to the cancer cell death if delivered to the tumor formation site. At this point, stem cells are used as vehicles for suicide gene transfer to tumors for the process called gene-directed enzyme/prodrug combination (GEPC) therapy. Stem cells are transduced with suicide genes such as cytosine deaminase (CD), carboxylesterase and/or herpes simplex virus thymidine-kinase by adenovirus, retrovirus or lentivirus.[41,42,57,67,68] These suicide genes effectively converts non-toxic prodrugs into their highly cytotoxic forms:[65,69,70] CD converts 5-fluorocytosine into 5-fluorouracil, carboxylesterase, CPT-11, SN-38 and herpes simplex virus thymidine-kinase, ganciclovir (GCV), active form of GCV.[62,64,67,69,71,72] For example, Gu *et al*.[73] evaluated the efficacy of the herpes simplex virus thymidine-kinase/GCV system using MSCs (MSCtk cells) in leptomeningeal glioma dissemination models. The results from this study showed that MSCtk cells exerted a strong 'bystander effect' both *in vitro* and *in vivo* conditions in the presence of GCV. Joo *et al*.[74] described the tumor-tropic properties of neural stem cells expressing CD suicide genes (HB1.F3.CD cells) and their ability to target brain tumors. Tumor burdens were significantly reduced in animal models with glioblastoma, medulloblastoma and metastatic neuroblastoma, demonstrating the tumor-targeting properties of the HB1.F3.CD cells in presence of the prodrug 5-fluorocytosine. HB1.F3.CD cells have been used for the development of a novel strategies for delivering therapeutic genes to tumors in the brain. The advantages in using stem cells as delivery vehicles for therapeutic gene transfer may be attributed to their specific tumor tropism. In the previous studies, stem cells were shown to effectively migrate to the cancer cells by reacting to the chemoattractant factors such as stem cell factor, vescular endothelial growth factor-2, C-X-C chemokine receptor type 4 and c-KIT expressed in cancer cells.[1,3,60] Meanwhile, the therapeutic potentials of amnion-derived stem cells in cancer gene therapy have been recently identified. According to our previous study, hAFCs engineered to express multiple suicide genes, CD and herpes simplex virus thymidine-kinase genes, were shown to significantly inhibit the growth of MDA-MB-231 human breast cancer cells *in vitro* and reduced the tumor volume of breast cancer xenografted mice *in vivo* in the presence of the prodrugs, 5-fluorocytosine and GCV.[75] Another recent study also reports that AF-MSCs engineered to express interferon-β can be adopted as therapeutic vehicles for the treatment of bladder cancer by the targeting of the tumor site and further by their high proliferation rate and expansion efficiency in culture.[76] Consequentially, these results indicate that amniotic-derived stem cells can serve as a vehicle in cell-based gene-directed enzyme prodrug system to selectively target malignant cancers. As the studies of engineered stem cells strategies using amnion-derived stem cells are in an early stage, more extensive trials are need to intensify their anticancer potential as therapeutic gene delivery vehicles.

CONCLUSIONS

Human amniotic membrane/fluid-derived stem cells possess a high proliferative potential, express the markers, that is, OCT4 specific to pluripotent stem cells, and have the potential to differentiate into cells of all three germ layers *in vitro*. Human amniotic-derived stem cells can be divided into three types: hAECs, AM-hMSCs and hAFCs. They are easily obtained from discarded amnions after parturition and their acquisition process is not affiliated with ethical issues. Furthermore, they have advantages when introduced to other bodies because they are non-tumorigenic and cause low immunogenicity and anti-inflammation. Anticancer potential of amniotic-derived stem cells suggests a new tool in the stem cell therapy. Novel therapies using hAECs and hAFCs include human stem cell transplantation with non-engineered stem cells and gene-directed enzyme/prodrug combination therapy with genetically engineered stem cells. In these strategies, amniotic-derived stem cells effectively target the tumor and suppressed the tumor growth by expressing various cytotoxic cytokines. Additionally, they also have a potential as novel delivery vehicles transferring therapeutic genes to the cancer formation sites in GEPC therapy. Taken together, the results of all studies mentioned above indicate that human amniotic membrane/fluid-derived stem cells may exert potent antitumor effects via their tumor-tropic and tumorcidal activities and can be a novel strategy to selectively target human cancers.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by two National Research Foundation of Korea (NRF) grants funded by the Ministry of Education, Science and Technology (MEST) of Korea Government (no. 2010-0003093 and 2011-0015385).

REFERENCES

1 Kim KY, Kim SU, Leung PC, Jeung EB, Choi KC. Influence of the prodrugs 5-fluorocytosine and CPT-11 on ovarian cancer cells using genetically engineered stem cells: tumor-tropic potential and inhibition of ovarian cancer cell growth. *Cancer Sci* 2010; **101**: 955–962.
2 Kim SU, Jeung EB, Kim YB, Cho MH, Choi KC. Potential tumor-tropic effect of genetically engineered stem cells expressing suicide enzymes to selectively target invasive cancer in animal models. *Anticancer Res* 2011; **31**: 1249–1258.
3 Yi BR, O SN, Kang NH, Hwang KA, Kim SU, Jeung EB *et al.* Genetically engineered stem cells expressing cytosine deaminase and interferon-beta migrate to human lung cancer cells and have potentially therapeutic anti-tumor effects. *Int J Oncol* 2011; **39**: 833–839.
4 Cao F, Lin S, Xie X, Ray P, Patel M, Zhang X *et al. In vivo* visualization of embryonic stem cell survival, proliferation, and migration after cardiac delivery. *Circulation* 2006; **113**: 1005–1014.
5 Xie X, Cao F, Sheikh AY, Li Z, Connolly AJ, Pei X *et al.* Genetic modification of embryonic stem cells with VEGF enhances cell survival and improves cardiac function. *Cloning Stem Cells* 2007; **9**: 549–563.
6 Chaudhry GR, Fecek C, Lai MM, Wu WC, Chang M, Vasquez A *et al.* Fate of embryonic stem cell derivatives implanted into the vitreous of a slow retinal degenerative mouse model. *Stem Cells Dev Mar* 2009; **18**: 247–258.
7 Lee AS, Tang C, Cao F, Xie X, van der Bogt K, Hwang A *et al.* Effects of cell number on teratoma formation by human embryonic stem cells. *Cell Cycle* 2009; **8**: 2608–2612.
8 Dobreva MP, Pereira PN, Deprest J, Zwijsen A. On the origin of amniotic stem cells: of mice and men. *Int J Dev Biol* 2010; **54**: 761–777.
9 Ayuzawa R, Doi C, Rachakatla RS, Pyle MM, Maurya DK, Troyer D *et al.* Naive human umbilical cord matrix derived stem cells significantly attenuate growth of human breast cancer cells *in vitro* and *in vivo*. *Cancer Lett* 2009; **280**: 31–37.
10 Gurudutta GU, Satija NK, Singh VK, Verma YK, Gupta P, Tripathi RP. Stem cell therapy: a novel & futuristic treatment modality for disaster injuries. *Indian J Med Res* 2012; **135**: 15–25.
11 Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. *Circ Res* 2007; **100**: 1249–1260.
12 Secchiero P, Zorzet S, Tripodo C, Corallini F, Melloni E, Caruso L *et al.* Human bone marrow mesenchymal stem cells display anti-cancer activity in SCID mice bearing disseminated non-Hodgkin's lymphoma xenografts. *PLoS One* 2010; **5**: e11140.
13 Klopp AH, Gupta A, Spaeth E, Andreeff M, Marini III F. Concise review: dissecting a discrepancy in the literature: do mesenchymal stem cells support or suppress tumor growth? *Stem Cells* 2011; **29**: 11–19.
14 Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW *et al.* Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature* 2007; **449**: 557–563.
15 Toda A, Okabe M, Yoshida T, Nikaido T. The potential of amniotic membrane/amnion-derived cells for regeneration of various tissues. *J Pharmacol Sci* 2007; **105**: 215–228.
16 Da Sacco S, Sedrakyan S, Boldrin F, Giuliani S, Parnigotto P, Habibian R *et al.* Human amniotic fluid as a potential new source of organ specific precursor cells for future regenerative medicine applications. *J Urol* 2010; **183**: 1193–1200.
17 De Coppi P, Bartsch Jr G, Siddiqui MM, Xu T, Santos CC, Perin L *et al.* Isolation of amniotic stem cell lines with potential for therapy. *Nat Biotechnol* 2007; **25**: 100–106.
18 Paracchini V, Carbone A, Colombo F, Castellani S, Mazzucchelli S, Gioia SD *et al.* Amniotic mesenchymal stem cells: a new source for hepatocyte-like cells and induction of CFTR expression by coculture with cystic fibrosis airway epithelial cells. *J Biomed Biotechnol* 2012; **2012**: 575471.
19 Tamagawa T, Ishiwata I, Nakamura Y. Differentiation of human amniotic membrane cells into osteoblasts *in vitro*. *Hum Cell* 2005; **18**: 191–195.
20 Takashima S, Yasuo M, Sanzen N, Sekiguchi K, Okabe M, Yoshida T *et al.* Characterization of laminin isoforms in human amnion. *Tissue Cell* 2008; **40**: 75–81.
21 Kobayashi M, Yakuwa T, Sasaki K, Sato K, Kikuchi A, Kamo I *et al.* Multilineage potential of side population cells from human amnion mesenchymal layer. *Cell Transplant* 2008; **17**: 291–301.
22 Kakishita K, Nakao N, Sakuragawa N, Itakura T. Implantation of human amniotic epithelial cells prevents the degeneration of nigral dopamine neurons in rats with 6-hydroxydopamine lesions. *Brain Res* 2003; **980**: 48–56.
23 Wu ZY, Hui GZ, Lu Y, Wu X, Guo LH. Transplantation of human amniotic epithelial cells improves hindlimb function in rats with spinal cord injury. *Chin Med J (Engl)* 2006; **119**: 2101–2107.
24 Jiao H, Guan F, Yang B, Li J, Song L, Hu X *et al.* Human amniotic membrane derived-mesenchymal stem cells induce C6 glioma apoptosis *in vivo* through the Bcl-2/caspase pathways. *Mol Biol Rep* 2012; **39**: 467–473.
25 Luo H, Huang X, Huang F, Liu X. [Preliminary study on transdifferentiation of human amniotic epithelial cells and its intrasplenic transplantation]. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi* 2011; **25**: 144–148.
26 Zhang D, Jiang M, Miao D. Transplanted human amniotic membrane-derived mesenchymal stem cells ameliorate carbon tetrachloride-induced liver cirrhosis in mouse. *PLoS One* 2011; **6**: e16789.
27 Simat SF, Chua KH, Abdul Rahman H, Tan AE, Tan GC. The stemness gene expression of cultured human amniotic epithelial cells in serial passages. *Med J Malaysia* 2008; **63**(Suppl A): 53–54.
28 Miki T, Lehmann T, Cai H, Stolz DB, Strom SC. Stem cell characteristics of amniotic epithelial cells. *Stem Cells* 2005; **23**: 1549–1559.
29 Marongiu F, Gramignoli R, Dorko K, Miki T, Ranade AR, Paola Serra M *et al.* Hepatic differentiation of amniotic epithelial cells. *Hepatology* 2011; **53**: 1719–1729.
30 Ilancheran S, Michalska A, Peh G, Wallace EM, Pera M, Manuelpillai U. Stem cells derived from human fetal membranes display multilineage differentiation potential. *Biol Reprod* 2007; **77**: 577–588.
31 Alviano F, Fossati V, Marchionni C, Arpinati M, Bonsi L, Franchina M *et al.* Term amniotic membrane is a high throughput source for multipotent mesenchymal stem cells with the ability to differentiate into endothelial cells *in vitro*. *BMC Dev Biol* 2007; **7**: 11.
32 Kim J, Lee Y, Kim H, Hwang KJ, Kwon HC, Kim SK *et al.* Human amniotic fluid-derived stem cells have characteristics of multipotent stem cells. *Cell Prolif* 2007; **40**: 75–90.
33 In 't Anker PS, Scherjon SA, Kleijburg-van der Keur C *et al.* Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation. *Blood* 2003; **102**: 1548–1549.
34 Zhao P, Ise H, Hongo M, Ota M, Konishi I, Nikaido T. Human amniotic mesenchymal cells have some characteristics of cardiomyocytes. *Transplantation* 2005; **79**: 528–535.
35 Phermthai T, Odglun Y, Julavijitphong S, Titapant V, Chuenwattana P, Vantanasiri C *et al.* A novel method to derive amniotic fluid stem cells for therapeutic purposes. *BMC Cell Biol* 2010; **11**: 79.
36 Prusa AR, Hengstschlager M. Amniotic fluid cells and human stem cell research: a new connection. *Med Sci Monit* 2002; **8**: RA253–RA257.
37 Prusa AR, Marton E, Rosner M, Bernaschek G, Hengstschlager M. Oct-4-expressing cells in human amniotic fluid: a new source for stem cell research? *Hum Reprod* 2003; **18**: 1489–1493.
38 Zhang X, Chen X, Wang H, Liu S. [Development of amniotic fluid-derived stem cell]. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi* 2008; **22**: 864–868.
39 Aboody KS, Bush RA, Garcia E, Metz MZ, Najbauer J, Justus KA *et al.* Development of a tumor-selective approach to treat metastatic cancer. *PLoS One* 2006; **1**: e23.
40 Aboody KS, Najbauer J, Schmidt NO, Yang W, Wu JK, Zhuge Y *et al.* Targeting of melanoma brain metastases using engineered neural stem/progenitor cells. *Neuro Oncol* 2006; **8**: 119–126.
41 Aghi M, Kramm CM, Chou TC, Breakefield XO, Chiocca EA. Synergistic anticancer effects of ganciclovir/thymidine kinase and 5-fluorocytosine/cytosine deaminase gene therapies. *J Natl Cancer Inst* 1998; **90**: 370–380.
42 Anderson LM, Krotz S, Weitzman SA, Thimmapaya B. Breast cancer-specific expression of the Candida albicans cytosine deaminase gene using a transcriptional targeting approach. *Cancer Gene Ther* 2000; **7**: 845–852.
43 Kucerova L, Matuskova M, Pastorakova A, Tyciakova S, Jakubikova J, Bohovic R *et al.* Cytosine deaminase expressing human mesenchymal stem cells mediated tumour regression in melanoma bearing mice. *J Gene Med* 2008; **10**: 1071–1082.
44 Lee DH, Ahn Y, Kim SU *et al.* Targeting rat brainstem glioma using human neural stem cells and human mesenchymal stem cells. *Clin Cancer Res* 2009; **15**: 4925–4934.
45 Fidan E, Fidan S, Yildiz B, Durmus I, Kavgaci H, Ozdemir F *et al.* Bolus fluorouracil induced syncope and pulseless ventricular tachycardia: a case report. *Hippokratia* 2011; **15**: 93–95.
46 Longley DB, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. *Nat Rev Cancer* 2003; **3**: 330–338.
47 Khakoo AY, Pati S, Anderson SA, Reid W, Elshal MF, Rovira II *et al.* Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi's sarcoma. *J Exp Med* 2006; **203**: 1235–1247.
48 Roland CL, Lynn KD, Toombs JE, Dineen SP, Udugamasooriya DG, Brekken RA. Cytokine levels correlate with immune cell infiltration after anti-VEGF therapy in preclinical mouse models of breast cancer. *PLoS One* 2009; **4**: e7669.

49 Ohlsson LB, Varas L, Kjellman C, Edvardsen K, Lindvall M. Mesenchymal progenitor cell-mediated inhibition of tumor growth *in vivo* and *in vitro* in gelatin matrix. *Exp Mol Pathol* 2003; **75**: 248–255.
50 Gardner SL. Application of stem cell transplant for brain tumors. *Pediatr Transplant* 2004; **8**(Suppl 5): 28–32.
51 Koskimaki JE, Karagiannis ED, Rosca EV, Vesuna F, Winnard Jr PT, Raman V *et al.* Peptides derived from type IV collagen, CXC chemokines, and thrombospondin-1 domain-containing proteins inhibit neovascularization and suppress tumor growth in MDA-MB-231 breast cancer xenografts. *Neoplasia* 2009; **11**: 1285–1291.
52 Qiao L, Xu ZL, Zhao TJ, Ye LH, Zhang XD. Dkk-1 secreted by mesenchymal stem cells inhibits growth of breast cancer cells via depression of Wnt signalling. *Cancer Lett* 2008; **269**: 67–77.
53 Cho JA, Park H, Kim HK, Lim EH, Seo SW, Choi JS *et al.* Hyperthermia-treated mesenchymal stem cells exert antitumor effects on human carcinoma cell line. *Cancer* 2009; **115**: 311–323.
54 Kang NH, Yi BR, Lim SY, Hwang KA, Baek YS, Kang KS *et al.* Human amniotic membrane-derived epithelial stem cells display anticancer activity in BALB/c female nude mice bearing disseminated breast cancer xenografts. *Int J Oncol* 2012; **40**: 2022–2028.
55 Manuelpillai U, Tchongue J, Lourensz D, Vaghjiani V, Samuel CS, Liu A *et al.* Transplantation of human amnion epithelial cells reduces hepatic fibrosis in immunocompetent CCl-treated mice. *Cell Transplant* 2010; **19**: 1157–1168.
56 Peterson SM, Iskenderian A, Cook L, Romashko A, Tobin K, Jones M *et al.* Human sulfatase 2 inhibits *in vivo* tumor growth of MDA-MB-231 human breast cancer xenografts. *BMC Cancer* 2010; **10**: 427.
57 Bi WL, Parysek LM, Warnick R, Stambrook PJ. *In vitro* evidence that metabolic cooperation is responsible for the bystander effect observed with HSV tk retroviral gene therapy. *Hum Gene Ther* 1993; **4**: 725–731.
58 Iankov ID, Msaouel P, Allen C, Federspiel MJ, Bulur PA, Dietz AB *et al.* Demonstration of anti-tumor activity of oncolytic measles virus strains in a malignant pleural effusion breast cancer model. *Breast Cancer Res Treat* 2010; **122**: 745–754.
59 Hajitou A, Trepel M, Lilley CE, Soghomonyan S, Alauddin MM, Marini III FC *et al.* A hybrid vector for ligand-directed tumor targeting and molecular imaging. *Cell* 2006; **125**: 385–398.
60 Yi BR, Hwang KA, Kang NH, Kim SU, Jeung EB, Choi KC. Antitumor therapeutic effects of cytosine deaminase and interferon-beta against endometrial cancer cells using genetically engineered stem cells *in vitro*. *Anticancer Res* 2011; **31**: 2853–2862.
61 Kucerova L, Altanerova V, Matuskova M, Tyciakova S, Altaner C. Adipose tissue-derived human mesenchymal stem cells mediated prodrug cancer gene therapy. *Cancer Res* 2007; **67**: 6304–6313.
62 Lifang Y, Min T, Midan A, Ya C. HSV-tk/GCV gene therapy mediated by EBV-LMP1 for EBV-associated cancer. *J Exp Clin Cancer Res* 2008; **27**: 42.
63 Ishii-Morita H, Agbaria R, Mullen CA, Hirano H, Koeplin DA, Ram Z *et al.* Mechanism of 'bystander effect' killing in the herpes simplex thymidine kinase gene therapy model of cancer treatment. *Gene Ther* 1997; **4**: 244–251.
64 Tang W, He Y, Zhou S, Ma Y, Liu G. A novel Bifidobacterium infantis-mediated TK/GCV suicide gene therapy system exhibits antitumor activity in a rat model of bladder cancer. *J Exp Clin Cancer Res* 2009; **28**: 155.
65 Uckert W, Kammertons T, Haack K, Qin Z, Gebert J, Schendel DJ *et al.* Double suicide gene (cytosine deaminase and herpes simplex virus thymidine kinase) but not single gene transfer allows reliable elimination of tumor cells *in vivo*. *Hum Gene Ther* 1998; **9**: 855–865.
66 Kim SK, Kim SU, Park IH *et al.* Human neural stem cells target experimental intracranial medulloblastoma and deliver a therapeutic gene leading to tumor regression. *Clin Cancer Res* 2006; **12**: 5550–5556.
67 Boucher PD, Im MM, Freytag SO, Shewach DS. A novel mechanism of synergistic cytotoxicity with 5-fluorocytosine and ganciclovir in double suicide gene therapy. *Cancer Res* 2006; **66**: 3230–3237.
68 Coley HM. Mechanisms and strategies to overcome chemotherapy resistance in metastatic breast cancer. *Cancer Treat Rev* 2008; **34**: 378–390.
69 Tomicic MT, Thust R, Kaina B. Ganciclovir-induced apoptosis in HSV-1 thymidine kinase expressing cells: critical role of DNA breaks, Bcl-2 decline and caspase-9 activation. *Oncogene* 2002; **21**: 2141–2153.
70 Yi C, Huang Y, Guo ZY, Wang SR. Antitumor effect of cytosine deaminase/5-fluorocytosine suicide gene therapy system mediated by Bifidobacterium infantis on melanoma. *Acta Pharmacol Sin* 2005; **26**: 629–634.
71 Rubsam LZ, Davidson BL, Shewach DS. Superior cytotoxicity with ganciclovir compared with acyclovir and 1-beta-D-arabinofuranosylthymine in herpes simplex virus-thymidine kinase-expressing cells: a novel paradigm for cell killing. *Cancer Res* 1998; **58**: 3873–3882.
72 Zhang JF, Wei F, Wang HP, Li HM, Qiu W, Ren PK *et al.* Potent anti-tumor activity of telomerase-dependent and HSV-TK armed oncolytic adenovirus for non-small cell lung cancer *in vitro* and *in vivo*. *J Exp Clin Cancer Res* 2010; **29**: 52.
73 Gu C, Li S, Tokuyama T, Yokota N, Namba H. Therapeutic effect of genetically engineered mesenchymal stem cells in rat experimental leptomeningeal glioma model. *Cancer Lett* 2010; **291**: 256–262.
74 Joo KM, Park IH, Shin JY, Jin J, Kang BG, Kim MH *et al.* Human neural stem cells can target and deliver therapeutic genes to breast cancer brain metastases. *Mol Ther* 2009; **17**: 570–575.
75 Kang NH, Choi KC. 207 Engineered human amniotic fluid-derived stem cells by expressing cytosine deaminase (cd) and thymidine kinase (hsv-tk) mediate targeting killing effect in breast cancer cells. *Reprod Fertil Dev* 2012; **24**: 216.
76 Bitsika V, Roubelakis MG, Zagoura D, Trohatou O, Makridakis M, Pappa KI *et al.* Human amniotic fluid-derived mesenchymal stem cells as therapeutic vehicles: a novel approach for the treatment of bladder cancer. *Stem Cells Dev* 2012; **21**: 1097–1111.
77 Hauser PV, De Fazio R, Bruno S, Sdei S, Grange C, Bussolati B *et al.* Stem cells derived from human amniotic fluid contribute to acute kidney injury recovery. *Am J Pathol* 2010; **177**: 2011–2021.