

REVIEW

Amnion-derived stem cells: in quest of clinical applications

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Abstract

In the promising field of regenerative medicine, human perinatal stem cells are of great interest as potential stem cells with clinical applications. Perinatal stem cells could be isolated from normally discarded human placentae, which are an ideal cell source in terms of availability, the fewer number of ethical concerns, less DNA damage, and so on. Numerous studies have demonstrated that some of the placenta-derived cells possess stem cell characteristics like pluripotent differentiation ability, particularly in amniotic epithelial (AE) cells. Term human amniotic epithelium contains a relatively large number of stem cell marker-positive cells as an adult stem cell source. In this review, we introduce a model theory of why so many AE cells possess stem cell characteristics. We also describe previous work concerning the therapeutic applications and discuss the pluripotency of the AE cells and potential pitfalls for amnion-derived stem cell research.

Introduction

The emerging field of regenerative medicine requires a reliable cell source in addition to biomaterial scaffolds and cytokine/growth factors. The 'cell' is a particularly critical element for cell replacement therapies in order to provide a safe and sufficient cell supply for clinical applications. Efforts to search for an adequate cell type and cell source have been conducted and have continued along with the discussions for their use in clinical application.

There are many potential cell sources for regenerative medicine, including bone marrow-derived mesenchymal stem cells, tissue-specific progenitor cells, embryonic

stem (ES) cells, and induced pluripotent stem (iPS) cells. Although their biological potentials have been demonstrated, none of these cells is widely accepted as a definitive cell source for clinical applications. Each cell type possesses different advantages as well as limitations for their use, such as safety or availability. It will be helpful to search for a potential stem cell source from the perspective of its potential for clinical application. What is the *sine qua non* of the cells for clinically applicable regenerative medicine? At the end of this review, this question will be discussed further.

There is increasing evidence that the human placenta contains pluripotent or multipotent stem cells or both. Various multipotent stem cells have been isolated from different parts of the human placenta, such as the amnion, chorion, umbilical cord, and fetal blood. As placenta-derived cells, these stem cells have common advantages (Figure 1). Specific types of placenta-derived stem cells, such as trophoblastic, hematopoietic, and mesenchymal stroma cells, have been discussed elsewhere [1-3]. Here, we will review stem cells derived from the amnion of human placentae, specifically amniotic epithelial (AE) cells. First, we will summarize previous studies that have demonstrated the unique stem cell characteristics of AE cells. On the basis of these findings, we introduce a model theory that explains why some AE cells, unlike other adult somatic stem cells, may possess pluripotent features. Second, we will discuss topics and pitfalls that are currently under discussion. Third, previous works that are leading the therapeutic application of AE cells will be summarized. Last, the potential of the clinical application of AE-derived stem cells and the future direction of the research are discussed.

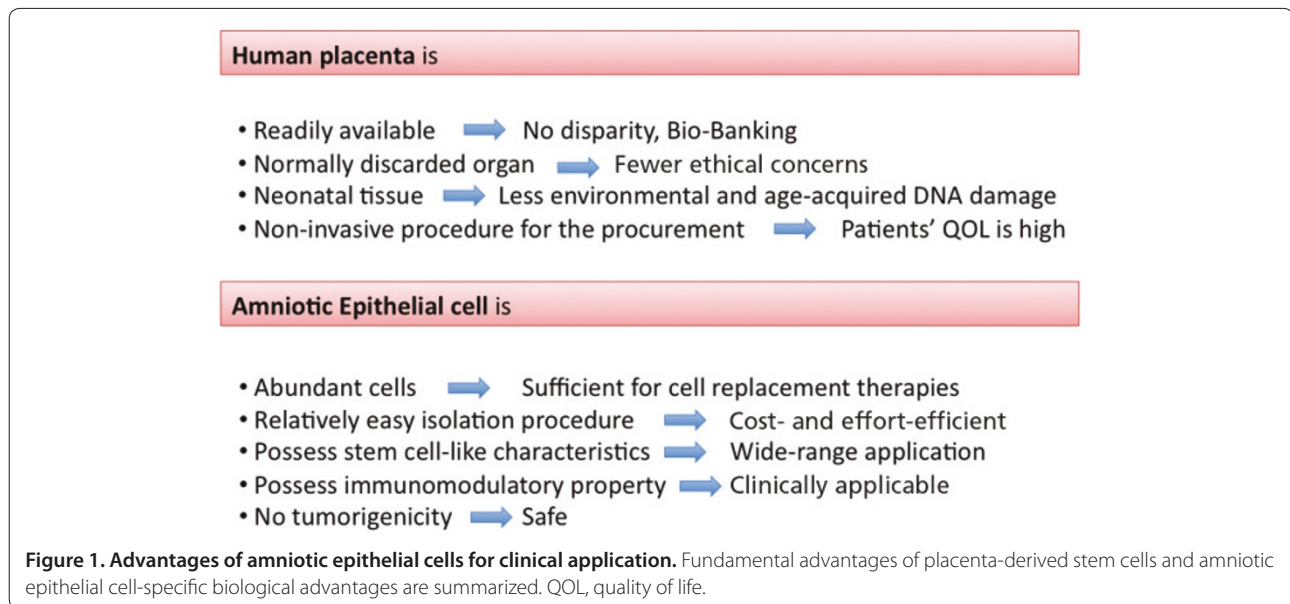
Amniotic epithelial cells: what is so special about them?

The epithelial cell population could be exclusively isolated from the amnions of term human placentae by specific enzymatic digestion [4]. The cell surface antigen profile data indicate that AE cells are basically homogeneous cell populations for most of the cell surface markers [5]; however, the reactivity against 'stem cell'-specific antigens varies. Following isolation, some of the

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AE cells express stem cell surface markers, such as stage-specific embryonic antigen-3 (SSEA-3) and SSEA-4 and tumor rejection antigen 1-60 (TRA1-60) and TRA1-81, which are known to be expressed on human ES cells [6]. About 15%, 50%, and 5% to 10% of naïve human AE (hAE) cells are positive for SSEA-3, SSEA-4, and TRA stem cell markers, respectively [7]. Normally, undifferentiated stem cells homogeneously express these stem cell markers [6]. The variance of the ratio of stem cell marker-positive cells indicates that naïve AE cell populations contain cells in various stage of 'stemness'. Interestingly, the ratios of stem cell marker-positive AE cells (5% to 50%) are considerably higher than for other somatic/tissue stem cells. Most of the somatic/tissue stem cells are 0.1% to 0.01% of the residing tissue. For instance, the hematopoietic stem cell population is only 0.01% to 0.05% of all bone marrow cells [8]. The relatively high ratio of stem cell marker-positive cells in AE cell populations as somatic stem cells could be explained by the model theory. The cell surface markers that are expressed by hAE cells are summarized and compared with the expression of other types of stem cells in Table 1 [2,7,9-15].

Stem cells 'left behind': developmental uniqueness of the amniotic epithelial cell

Unlike other parts of the placenta, the amniotic epithelium is a tissue of epiblastic origin. Human amnioblast is derived from the pluripotent epiblast around the eighth day following fertilization, whereas other parts of the placenta are derived from the trophoctoderm. When the blastocyst is partially embedded in the endometrial stroma, the inner cell mass (or embryoblast) differentiates

into two layers: the hypoblast and the epiblast. The epiblast is the source of all three germ layers and eventually forms the developing embryo. At the same time, a small cavity (amniotic cavity) appears within the epiblast. Epiblast cells adjacent to the amniotic cavity (Figure 2) are called amnioblasts, which eventually form the amniotic epithelium. Concomitantly, some of the migrating hypoblasts transdifferentiate into mesenchymal cells (extraembryonic mesoderm) and develop into the amniotic connective tissue. The epiblast-amnioblast segregation occurs before gastrulation, which is considered the first dynamic event of organogenesis. All short-range organogenetic signals may not reach the segregated stem cells throughout gestation. For instance, cardiogenesis is a complex event that is orchestrated by short-range fibroblast growth factors (FGFs) and Hedgehog signals [16]. Therefore, some epiblasts/amnioblasts that are spatially segregated by the amniotic cavity from the epicenter of organogenesis may escape from these differentiation cues. After 10 months, although most of the cells have differentiated by following the epithelial cell fate and have lost their stem cell characteristics, about 5% to 10% of the AE cells may retain the epiblast-like stem cell characteristics at term [7]. If this model theory is correct, fetal amniotic epithelium should contain more stem cell marker-positive cells than term amniotic epithelium. Izumi and colleagues [17] demonstrated that about 40% and 30% of fetal (early second trimester) AE cells are positive for stem cell markers TRA1-60 and TRA 1-81, respectively, whereas 5% of term AE cells are positive for these markers. The amnion is a fairly large tissue that may not be very uniform but is rather regionalized [18]. To exclude variances due to the

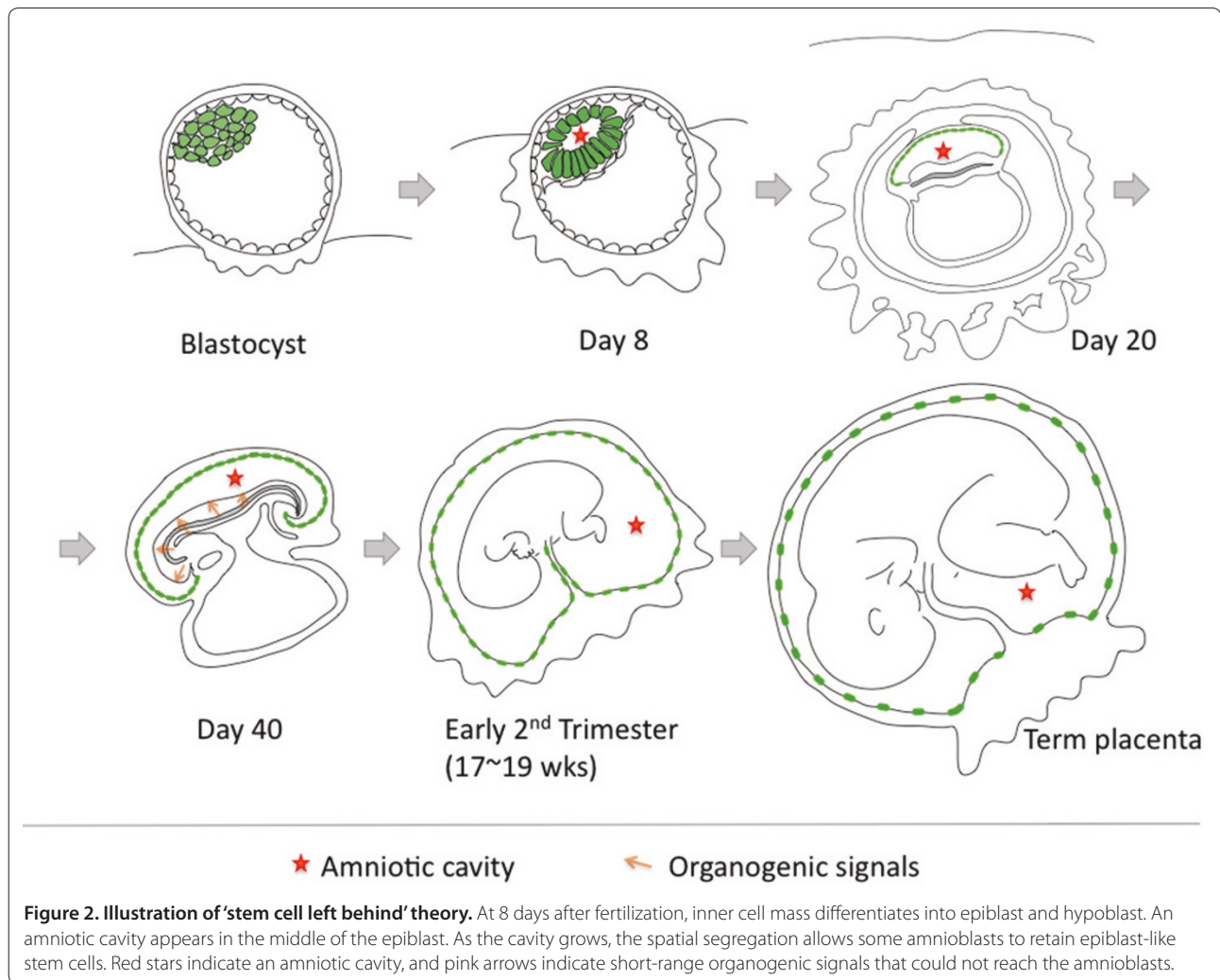
Table 1. Comparison of stem cell surface marker expression in stem cells

First author	AE								ES Osman	NS	MS	HS
	Fatimah	Bilic	Stadler	Banas	Parolini	Minas	Ilancheran	Miki				
Reference	[9]	[10]	[11]	[12]	[2]	[13]	[14]	[5,7]	[15]			
c-met				+							+	
CCR4				-				±	+			
CD10					+						+	
CD105			Up		+						+	
CD106 (VCAM-1)								-			+	
CD117 (c-kit)	-			-	±			±			+	+
CD13			Up		+						+	
CD14					-							
CD133				-				-	+	+	+	+
CD140b					+							
CD166 (ALCAM)		+			+						+	
CD24				+				+	+	+		
CD29				+	+				+		+	
CD31 (PECAM-1)	-							-			-	
CD324 (E-cadherin)				+	+			+	+			
CD338 (ABCG2)				+				+	+	+		+
CD34	-	-		-	-			-			-	+
CD349					-							
CD44	+	+	Up		+						+	
CD45	-	-			-						-	
CD49d					-							
CD49e		+	Up		+							
CD49f				+				+	+			
CD54 (ICAM-1)			+					+			+	
CD73	+	+			+						+	
CD9	+			+				+	+	+		
CD90	+		Up	Up	+		+				+	
GCTM2					+	+			+			
Sialyl Lewis a					+							
SSEA-1				-	-		-	-	-			
SSEA3		+		+	+		+	+	+			
SSEA4		+	+	+	+	+	+	+	+			
TRA1-60			Down	+	+		+	+	+			
TRA1-81			Down	+	+		+	+	+			

-, negative; +, positive; ±, weak; AE, amniotic epithelial cell; Down, downregulated each antigen expression; ES, embryonic stem cell; HS, hematopoietic stem cell; MS, mesenchymal stem cell; NS, neural stem cell; SSEA, stage-specific embryonic antigen; TRA, tumor rejection antigen; Up, upregulated each antigen expression.

regionalized stem cell localization, amnion samples were harvested from three different parts of the amnion: the center of the disc, the edge of the disc, and the membrane part. There was no significant difference between samples by region, at least in these three parts [19]. On the other hand, the mechanism and signals that induce differentiation on 90% of amnioblasts of epiblast origin are

unclear. It has been shown that cultured AE cells secrete various morphogens and growth factors such as epidermal growth factor, Noggin, Activin [20], platelet-derived growth factor, vascular endothelial growth factor, angiogenin, transforming growth factor-beta-2 (TGF-β2), and tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2) [21]. In addition to playing an important role in



maintaining pregnancy, these factors may induce AE cell maturation or apoptosis to the epiblast-like immature AE cells. It must be noted that hAE cells are able to support the pluripotency of primate and mouse ES cells when primary hAE cells were used as feeder layer cells [22,23]. These data indicate that some of the secreted factors or cell-to-cell signaling (or both) might play a role in maintaining epiblast-like stemness of some AE cells. There are, however, no clear experimental data that indicate why the stem cell marker-positive AE cells unevenly differentiate even though all AE cells are exposed to the same environmental signals from the amniotic fluid. One of the possible mechanisms is 'lateral inhibition,' which is a type of cell-to-cell interaction to regulate cell fate in the development of various cell types. This could be an interesting question for further investigation.

Amniotic epithelial cells possess pluripotency?

In addition to expressing stem cell-specific surface markers, AE cells express molecular markers of pluripotent stem

cells: octamer-4 (*OCT-4*), *NANOG*, sex determining region Y-box 2 (*SOX-2*), *Lefty-A*, *FGF-4*, *REX-1*, and teratocarcinoma-derived growth factor 1 (*TDGF-1*) (*cripto-1*). Among those molecular stem cell markers, *OCT-4* is known as one of the transcription factors that play a critical role in maintaining pluripotency and self-renewal. *OCT-4* belongs to the POU family of transcriptional regulators [24-26] and regulates the pluripotency of human and mouse ES cells [27]. Expression of *OCT-4* is decreased along with the stem cell differentiation and the loss of expression leading to differentiation [28]. At the epiblast stage, *OCT-4* continues to be expressed as long as cells remain undifferentiated [26]. The expression of *OCT-4* is controlled epigenetically by hypermethylation of the enhancer/promoter region [29].

OCT-4 protein expression is observed in most AE cells. Some display nuclear-localized *OCT-4*, but for the majority of AE cells, the expression is cytoplasmic. There is concern over the *OCT-4* expression in somatic cells [30]. *OCT-4* exists as two splice variants: *OCT-4A* and

OCT-4B [31]. Recent studies have suggested that it is the OCT-4A isoform that has the ability to confer and sustain pluripotency but that the OCT-4B may not be functional [32,33]. Lengner and colleagues [34] pointed out that published data describing positive results of OCT-4 expression in somatic stem cells might be erroneous because of investigator ignorance of the two isoforms. Primers or antibodies that recognize both isoforms might be misused to claim functional OCT-4 expression in some somatic stem cells. We have confirmed OCT-4A expression in naïve hAE cells by using a commercially available primer and probe set (Hs0300511_g1; Applied Biosystems, Foster City, CA, USA) that matches OCT-4A-specific exons [17].

Although a number of investigations have provided evidence that suggests multipotency of AE cells, the pluripotency has not yet been proven. One of the critical issues is the difficulty to establish clonal expansion from a single AE cell, a step that is essential to demonstrate pluripotency *in vitro*. Unlike mouse ES cells, human ES cells and mouse epiblast-derived stem (EpiSC) cells are intolerant to passaging as single cells. Like EpiSC cells, AE cells do not maintain their stem cell characteristics well or survive as a single cell in culture. AE cells easily fall into the senescence state or differentiate into palm-shaped epithelial cells when cultured at low density. The teratoma formation assay has been used as a gold standard assay to prove pluripotency of ES or iPS cells. However, this assay cannot be applied to evaluate AE cells. Because of the genetically stable characteristics, the AE cell does not form a teratoma when injected into immunodeficient mice [7,14]. The ultimate approach to determine pluripotency of AE-derived stem cells is generating chimeric animals. If an AE cell that is injected into the blastocyst will contribute to all germ layer cells in the resulting chimeric embryo, the pluripotency will be confirmed. In 2004, Tamagawa and colleagues [35] derived cell lines from human amnion and mixed them with mouse early embryonic stem cells to form an aggregation chimera. The authors succeeded in demonstrating that the human cells contributed to all three primordial germ layer formations in the xenogeneic chimera embryo [35]. Although the cell line cells are established from a mixed amniotic cell population that contains both AE cells and amniotic mesenchymal fibroblasts, this investigation suggested the pluripotency of the human amniotic cells. Further investigation will be required to clarify which cell population is responsible for the pluripotency.

Multipotency of amniotic epithelial cells and the therapeutic potential

Although the pluripotency of a single AE cell is not clarified yet, AE cells differentiate into cells of all three

germ layers under appropriate culture conditions [7,14]. The changes of gene expression and cell morphology of AE cells in these experiments demonstrated the AE cell plasticity that is induced by exposure to exogenous growth factors or chemicals. At present, it has not been confirmed whether a single pluripotent amniotic stem cell differentiates into all three germ layers or whether there are various lineage-committed multipotent cells in the AE cell population. In spite of this critical question from a basic science point of view, it is a secondary concern from the perspective of clinical application. Since it is impossible to simultaneously induce the desired differentiation in 100% of the starting material of stem cells, some form of purification process is essential prior to using stem cell-derived therapeutic cells in clinical application. Therefore, the most important question from a clinical point of view is whether therapeutically useful cells can be produced from the hAE cell population. Here, we summarize previous works that suggest the differentiation potential of AE cells and the therapeutic potential tested in animal models.

It must be noted that there are developmental and anatomical differences between rodent and human amnion. Rodent amniotic epithelium is clearly derived from epiblasts; therefore, the usage of rodent AE cells as a model could be appropriate. However, owing to the size and anatomical uniqueness, the isolation of AE cells must be done very carefully. Recently, Dobrova and colleagues [36] focused on the species differences of the amnion and comprehensively reviewed this topic. This review is strongly recommended to researchers who plan to conduct research with rodent amnion stem cells, including amniotic fluid-derived stem cells.

Ectoderm lineage

Neurodegenerative diseases are among the most suitable target diseases for stem cell-based therapies. Since neurodegenerative diseases have many pathological processes in common, the cell transplantation approach could potentially ameliorate the symptoms of several distinct neurodegenerative diseases. There are two expected mechanisms of cell transplantation. One is the differentiation potential of the transplanted stem/progenitor cells to neural cells. Sakuragawa's group [37], pioneers in AE research, demonstrated that cultured AE cells express markers of glial and neuronal progenitor cells. Our group confirmed that naïve hAE cells express various neural marker genes, including neuron-specific enolase, neurofilament-M, myelin basic protein, microtubule-associated protein 2, and glial fibrillary acid protein [7]. Under appropriate culture conditions, AE cells express or upregulate neuron-specific gene expressions such as nestin and glutamic acid decarboxylase. Using the adenoviral labeling system, Ishii and colleagues

[38] demonstrated that about 20% of hAE cells express oligodendrocyte marker genes, myelin basic protein, proteolipid protein, and 2',3'-cyclic nucleotide 3'-phosphodiesterase (*CNPase*). The neural differentiation capabilities of AE cells were confirmed by various researchers [39,40]. Amazingly, the capabilities were preserved even after long-term cryopreservation. Human amniocytes that were originally isolated in 1974 adopted neuronal morphology and expression of neural genes, including β -III-tubulin, *Gap-43*, *NF-M*, *TAU*, and synaptophysin, after more than 30 years [41].

The other expectation of cell transplantation for neurodegenerative diseases is the ability to secrete functional or protective factors from the transplanted cells such as dopamine or some other factors, which result in protective/trophic effects or immunomodulatory effects [42,43]. For instance, in Parkinson's disease, there is a loss of the dopaminergic neural population in the substantia nigra [44]. In clinical settings, it has been shown that dopamine-producing tissue (fetal mesencephalic grafts) transplantation could ameliorate the symptoms [45]. hAE cells synthesize and release dopamine [46,47]. The dopamine synthesis responds to supplemented L-DOPA (L-3,4-dihydroxyphenylalanine) concentration in a dose-dependent manner. Furthermore, transplanted AE cells might release neuroprotective factors or induce neurogenesis to improve diseased or damaged environments or both. It has been shown that hAE cells produce and secrete various types of trophic factors such as nerve growth factor, neurotrophin-3, and brain-derived neurotrophic factor [48-51].

This neural differentiation and neurotrophic potential of hAE cells has been tested in animal models. Transplanted hAE cells alleviated Parkinson-like symptoms in a dopamine-denervated rat model [52]. In these experiments, the engrafted hAE cells showed paracrine or neurotrophic effects rather than a contribution via neural differentiation. However, when rat AE cells were transplanted into ischemic hippocampus of adult gerbils, the rat AE-derived neuron-like cells were observed after 5 weeks of the transplantation [53]. The neural differentiation and the therapeutic effect of AE cells were also tested in a rat stroke model. The transplanted hAE cells migrated to the ischemic area and reduced infarct volume and improved behavioral function [54]. Recently, Suh [55] reported that hAE cell transplantation restored memory function in a transgenic mouse model of Alzheimer's disease. Although the mechanism is under investigation, these data encourage the clinical applications of the hAE cells for neurodegenerative diseases.

Endoderm lineage

Two cell types, hepatocytes and insulin-producing pancreatic cells, are most desired among the endoderm

lineage cells. Both cells have been used for cell replacement therapies and their therapeutic concept and efficiency have been shown [56-59]. The insufficient supply of human hepatocytes or beta cells, however, is one of the reasons that prevent these promising therapies from becoming standard clinical applications. Thus, safe and constant supplies of these functional cells are urgently required. In addition, stem cell-derived hepatocytes will be useful not only for cell replacement therapy (hepatocyte transplantation) [57] but also for toxicology and drug development [60].

Sakuragawa and colleagues [61] reported that cultured hAE cells expressed and produced albumin and α -feto-protein *in vitro* and *in vivo*. The hepatic characteristics of hAE cells were extensively investigated by Takashima and colleagues [62]. Our group applied a step-wise exogenous growth factor stimulation protocol to induce further hepatic maturation in hAE cells [63]. The AE-derived hepatocyte-like cells expressed late-phase hepatic differentiation markers, including various inducible cytochrome P450 genes, which are essential for drug metabolism as functional hepatocytes. These cells were also transplanted into immunodeficient mice, and human α -1 antitrypsin was detected circulating in the serum of recipient mice, and this confirmed that the engrafted hAE cells function as hepatocytes in mouse liver. Recently, Manuelpillai and colleagues [64] transplanted hAE cells into drug-induced cirrhosis model animals and demonstrated the anti-fibrosis effect of hAE cells. The data indicate that the therapeutic effect of transplanted hAE cells is more likely the immunomodulatory effect by suppressing inflammatory activation of hepatic stellate cells. On the other hand, the authors demonstrated human albumin in mouse sera that might be secreted from differentiated hAE-derived hepatic cells. Rat amniotic cells have been isolated and used to simulate allogeneic cell transplantation [65-67]. The transplanted rat AE cells survived in the liver following allogeneic transplantation for at least 30 days [65]. Although the rodent amniotic cell property might be different from that of humans, the therapeutic efficiency of AE cells, together with the basal advantages of the placenta-derived stem cells, suggests a treatment option for liver diseases.

Several groups demonstrated the pancreatic differentiation potential of hAE cells [7,68,69]. Under appropriate culture conditions, pancreatic cell-related genes such as *PDX-1*, *PAX-6*, *NKX2.2*, insulin, and glucagon were up-regulated *in vitro* [7]. The therapeutic potential was also demonstrated by the transplantation of cultured hAE cells in the spleen of diabetic mice. The serum glucose levels were normalized for several months after the transplant, suggesting that the transplanted AE cells differentiated into insulin-producing beta cells [68]. This finding was later confirmed with comprehensive analyses

that demonstrated glucose-responsive c-peptide production [69]. *In vitro* differentiation and involvement of histamine nicotinamide-induced pancreatic differentiation were further investigated [70].

In addition to the hepatic and pancreatic differentiation, the capability of AE cells differentiating into other types of endoderm lineage cells has been reported. Moodley and colleagues [71] demonstrated that naïve human amnion epithelial cells differentiate into lung epithelium (type II pneumocyte) 2 weeks after parenteral injection into a bleomycin-induced lung injury SCID (severe combined immunodeficiency) mice model. The transplanted hAE cells reduced inflammation and abrogated fibrosis post-lung injury. Moritoki and colleagues [72] systemically transplanted EGFP (enhanced green fluorescent protein)-transgenic mice AE cells into chemically induced cholestasis mouse model animals. The EGFP and cholangiocyte marker CK7 double-positive cells formed a bile duct-like tubular structure in the chronic cholestatic mouse liver.

Mesoderm lineage

Because adult cardiomyocytes do not regenerate sufficiently, there is great interest in finding suitable cell sources for cellular cardiomyoplasty. hAE cells also possess the potential to differentiate into cardiac cells [7]. Although AE-derived cardiomyocyte-like cells expressed cardiac differentiation marker genes, immunocytochemistry analysis showed that the expression pattern of α -actinin was similar to that of immature cardiomyocytes. The therapeutic potential was demonstrated by using rat amniotic cells and a rat acute infarction model [73]. Although transplanted rat amniotic cells dramatically improved the cardiac function, only a few transplanted cells were differentiated into cardiomyocytes (α -actinin-positive cells). The therapeutic effect was speculated to be due to paracrine or immunomodulatory effects of the rat amniotic cells. An interesting application of amniotic membrane was tested, and the therapeutic efficiency was demonstrated. Cargnoni and colleagues [74] applied a fragment of human amniotic membrane as a cardiac patch on an infarction area of a rat heart. The post-ischemic cardiac function was significantly improved with the amnion patch. This investigation importantly demonstrated that secondary cardiac ischemic injury could be prevented by humoral factors that are released from the amnion. Recently, functional cardiac differentiation of human amniotic cells was demonstrated [75]. The cardiomyogenic differentiation was induced by a co-culture system with murine fetal cardiomyocytes. The structure of sarcomeric α -actinin and the spontaneous beating and *in vivo* contribution of human amnion-derived cardiomyocytes were demonstrated. Stem cell-derived cardiomyocytes are also expected to be

an important new tool for drug development [60]. The *in vitro* functional hAE-derived cardiomyocytes could be a cell source for these assays. Further investigation for culture condition optimization or direct reprogramming will be required along with a definition of selection markers of functional mature cardiomyocytes. The studies that demonstrate the differentiation capability of AE cells into all three germ layer lineages are summarized in Table 2.

Advantages of human amniotic epithelial cells for clinical applications

From the view of clinicians and patients, the *sine qua non* of clinically applicable stem cells is first, 'safety'; second, 'therapeutic efficiency'; and, last, 'availability/sufficient quantity'. Several types of stem cells could serve as cell sources for cellular therapy. Generally, stem cells are classified according to their differentiation ability and origin. Pluripotent stem cells, such as ES cells and iPS cells, are considered to be the most promising stem cells because of their tremendous differentiation ability. The 'safety', however, is always a concern. The pluripotency comes with genetical instability, which leads to concerns for tumorigenicity. Although the 'therapeutic efficiency' is promised, the long-term efficiency has not yet been proven. Furthermore, the expansion and maintenance to obtain a therapeutically sufficient number of cells require time, effort, and cost.

Adult stem cells can be derived from virtually any tissue or organ. Most adult stem cells are tissue-specific lineage-committed multipotent cells. Some adult stem cells such as mesenchymal stem cells and hematopoietic stem cells are already applied in clinics and showed therapeutic efficiency, mainly with their immunomodulatory property. Therefore, the clinical applications are considerably safe, particularly in the case of autologous transplantation.

A similar immunomodulatory property has been demonstrated with hAE cells [76-80]. hAE cells inhibited allogeneic mixed lymphocyte reactions in a dose-dependent manner with 66% to 93% inhibition [81]. Most of the report demonstrated the immunomodulatory effect by secretion of suppressive mediators such as TNF- α , FasL, TRAIL, TGF- β , and MIF. On the other hand, Banas and colleagues [12] demonstrated that the immunomodulatory effect of AE cells is dependent on cell-to-cell contact with responding T cells. By using non-serum culture conditions, the authors demonstrated that hAE cells inhibit peripheral blood mononuclear cell proliferative responses to mitogen, alloantigen, and recall antigen but preactivated T-cell blast response. The results suggested that the presence of HLA-G immunological cell surface molecules is responsible for the cell-to-cell immunosuppressive properties of AE cells. In addition to

Table 2. Differentiation potential of amniotic epithelial cells

Lineage	Cell type	Species	First author	Reference
Ectoderm	Neural progenitor cell	Human	Sakuragawa	[37]
	Dopamine-producing cell	Human	Kakishita	[47,52]
	Neural cell	Human	Miki	[7]
	Oligodendrocyte	Human	Ishii	[38]
	Neural cell	Human	Niknejad	[40]
	Neural cell	Human	Woodbury	[41]
	Neuronal	Rat	Okawa	[53]
	Neural	Rat	Marcus	[67]
Mesoderm	Adipogenic	Human	Ilancheran	[14]
	Chondrogenic	Human	Ilancheran	[14]
	Osteogenic	Human	Stadler	[11]
	Cardiomyogenic	Human	Miki	[7]
	Cardiomyogenic	Human	Tsuji	[75]
	Cardiomyogenic	Rat	Fujimoto	[73]
Endoderm	Hepatic	Human	Miki	[7,63]
	Hepatic	Human	Sakuragawa	[61]
	Hepatic	Human	Takashima	[62]
	Hepatic	Human	Manuelpillai	[64]
	Hepatic	Rat	Nakajima	[65]
	Hepatic	Rat	Takahashi	[66]
	Hepatic	Rat	Marcus	[67]
	Insulin-producing cell	Human	Miki	[7]
	Insulin-producing cell	Human	Wei	[68]
	Insulin-producing cell	Human	Hou	[69]
	Insulin-producing cell	Human	Szukiewicz	[70]
Bile duct	Mouse	Moritoki	[72]	
Pneumocyte	Human	Moodley	[71]	

the HLA-G expression [82-84], the expressions of complement inhibitory proteins, CD59 antigen, decay-accelerating factor, membrane attack complex, and Fas antigen/CD95/APO1 have been reported as potential immunoregulatory factors from hAE cells [85-88]. Nevertheless, further investigation is required to fully elucidate the underlying mechanisms of the immunomodulatory effect of hAE cells.

Importantly, the safety of AE upon transplantation has been shown in a clinical setting. hAE cells have been used in clinics to correct lysosomal storage disease [89-91]. Although the applications were not conducted as a stem cell therapy, more than 50 cases of AE cell/tissue transplantations have been performed in various institutes [90-92]. No tumor formation has been reported from these clinical trials. As it has been described, AE cells are clearly non-tumorigenic when transplanted into immunodeficient animals [7,14]. A total of one to two million hAE cells was injected into more than 50 individual mice,

which were observed for a maximum of 516 days. None of the AE cell transplants has led to the development of tumors by any route of administration in SCID-beige mice or Rag-2 knockout mice. In parallel, cytogenetic analysis confirmed genetical stability of cultured AE cells [7]. AE cells do not express telomerase reverse transcriptase (*TERT*) mRNA [7]. A study demonstrated that immortalized cells by expression of TERT could exhibit some neoplastic transformation toward what seem to be cancer stem cells [93]. Missing TERT expression may be a safety advantage. Since more than 100 million cells can be isolated from one placenta, long-term culture and massive replication are not required to use AE cells as a cell source. For instance, only half a million cells will be sufficient to improve the devastating symptoms of Parkinson's disease and Huntington's disease [94,95]. Furthermore, human placenta is a neonatal tissue that has less age-acquired and environmental DNA damage. Naturally, the neonatal cells should possess a life-long

viability. Given these facts, the AE cell is clearly a safe cell for clinical application.

A placenta is discarded after every live birth; objections to derive stem cells from placentae are not expected. Isolating and using stem cells from discarded human tissue are ultimate recycling biotechnologies, which would be acceptable in today's society. Human placenta is readily available tissue wherever human society exists. Therefore, there will be no regional disparities for placenta-derived stem cell therapies. Current statistics indicate that there are over 4 million births and over 1 million cesarean sections performed in the US every year. The sheer volume of available placenta tissue leads us to the idea of establishing a biobank system for placenta-derived stem cells. Nakatsuji and colleagues [96] estimated that a cell bank with only 30 stem cell lines could match the HLA-A, HLA-B, and HLA-DR haplotypes in 82.2% of the Japanese population. On the basis of these estimations and the theoretical number of available placentae, it is clearly feasible to establish a biobank that stores human placenta-derived stem cells with all HLA haplotypes. Clinically relevant stem cells should be easily and reproducibly cultured and manipulated. The AE cell isolation procedure is relatively easy and does not require a special laboratory set-up [4]. The biobanking system therefore could be established in any country and connected as a network to provide all HLA types, including race dominant types.

Ethical issues surrounding both embryonic and fetal stem cells do not apply to the use of discarded human placentae. However, once the therapeutic efficiency of placenta-derived stem cells is demonstrated, this normally discarded medical waste may turn into valuable property. The cell-acquiring process, cost, and proprietary rights will be new ethical issues. A regulatory-compliant system will be required for cell-acquiring and -providing processes. The precedent and current regulations for the umbilical cord blood cell usage could be useful to prepare a guideline for procurements of placenta-derived stem cells [97].

The use of this ideal stem cell could take one of two directions in future clinical regenerative medicine. One direction will be in developing technology to derive pluripotent stem cell lines from AE cells, which possess a biological potential equivalent to that of ES cells and iPS cells. This direction, however, may eliminate the AE cell advantage that is discussed here. The other direction is to aim directly at differentiation to obtain functional target cells. The stem cell-derived cell therapy requires a selection step prior to the cell application to patients to ensure safety and efficiency. The genetic stability and non-tumorigenicity of AE cells will be the advantage for this approach. Unlike in ES cells or iPS cells, leakage concerns at the selection step will not be a critical issue.

Conclusions

Herein, we reviewed the stem cell characteristics of amnion cells, especially AE cells. We introduced a model theory that may explain why so many cells with stem cell features are present in the amnion. The model theory has been proposed by several research teams, including ours [5,7,14,34]. Previous studies that demonstrate the differentiation and therapeutic potential of AE cells were summarized. We described four major reasons why placenta-derived cells are a significant cell source for clinical applications. The AE cell meets two important conditions that are required for clinically relevant stem cells: safety and availability. So far, no stem cells are able to differentiate into therapeutically useful cell types *in vitro*, or their differentiation is not well controlled. As with other types of stem cells, further investigations will be required to induce AE cells to differentiate into therapeutically useful cells. Since AE cells are extremely safe and show therapeutic efficiency in animal models, clinical application should be considered in the near future.

Abbreviations

AE, amniotic epithelial; EGFP, enhanced green fluorescent protein; EpiSC, epiblast-derived stem; ES, embryonic stem; FGF, fibroblast growth factor; hAE, human amniotic epithelial; HLA, human leukocyte antigen; iPS, induced pluripotent stem; OCT-4, octamer-4; SCID, severe combined immunodeficiency; SSEA, stage-specific embryonic antigen; TERT, telomerase reverse transcriptase; TRA, tumor rejection antigen.

Competing interests

The author owns stock in Stemnion, Inc. (Pittsburgh, PA, USA). He has received no payment for the preparation of this manuscript and declares that he has no other competing interests.

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