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HUMAN PLACENTA-DERIVED STEM CELLS - RECENT FINDINGS BASED ON THE MOLECULAR SCIENCE

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Abstract

The human placenta is a complex, multifunctional transient fetomaternal organ. The placenta is composed of the maternal decidua basalis and its fetal part, consisting of the mesenchymal and trophoblast cell lineages. Both the placenta and the amniotic membranes are abundant in readily available placenta-derived mesenchymal stem cells (PD-MSCs). The clinical application of the PD-MSCs opens new perspectives for regenerative medicine and the treatment of various degenerative disorders. Their properties depend on their paracrine activity – the secretion of the anti-inflammatory cytokines and specific exosomes. In contrast to the PD-MSCs, the trophoblast stem cells (TSCs) are much more elusive. They can only be isolated from the blastocyst-stage embryos or the first-trimester placental tissue, making that procedure quite demanding. Also, other cultures require specific, strictly controlled conditions. TSCs may be potentially used as an *in vitro* model of various placental pathologies, facilitating the elucidation of their mysterious pathogenesis and creating the environment for testing the new drug efficiency. Nonetheless, it is unlikely that they could be ever implemented as a part of novel cellular therapeutic strategies in humans.

Running title: Current knowledge on the placental stem cells

Keywords: human embryonic and fetal development, placenta-derived mesenchymal stem cells, placenta, trophoblast stem cells

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Introduction

The placenta is a complex fetomaternal organ build of maternal decidua basalis and its fetal part, which is composed of trophoblast cells, as well as the blood vessels and fetal connective tissue. Trophoblast cells are divided into three major cellular populations syncytiotrophoblast (ST), cytotrophoblast (CT), and extravillous cytotrophoblast cells (EVT) [1]. The placenta regulates multiple physiological processes in human pregnancy. Its activity depends on the function of various cellular subpopulations, which have different characteristic properties. ST and CT cells form the structure of placental villi. They are responsible for the transfer of respiratory gases and energy substrates across the placental barrier and the elimination of the metabolic wastes from the fetal circulation. Moreover, from the middle part of the pregnancy, ST cells serve as a substantial origin of gestational hormones such as progesterone and human chorionic gonadotropin [2]. Abnormal placentation leads to the development of numerous gestational complications. EVT cells are mainly involved in the regulation of the invasiveness of the placenta. They penetrate the maternal decidua basalis and promote the invasion and remodeling of uterine spiral arteries to provide the optimal fetomaternal blood flow [3].

The term placenta and amniotic membranes are a rich source of multipotent mesenchymal stem cells (MSCs) that are capable of differentiating into multiple cell lines that have a potential to form numerous types of connective tissue (e.g., bone, cartilage, tendon, ligament, muscle, or the adipose tissue) [4,5]. In general, the placental tissue after the delivery is treated as common medical waste. Considering that, the potential source of the placenta-derived mesenchymal stem cells (PD-MSCs) is relatively easy to obtain. Due to the advances in laboratory techniques that have been made in recent years, both their isolation and the subsequent culture protocols are relatively simple [6]. The allogeneic PD-MSCs are thought to be a promising tool for clinicians, especially in regenerative medicine and the treatment of degenerative disorders. PD-MSCs and other MSCs could directly differentiate into specific cells in damaged tissues and facilitate their regeneration through the secretion of various molecules such as growth factors and cytokines [7].

Besides the mesenchymal stem cells, the early developing placental tissue is the origin of the population of trophoblast stem cells (TSCs) [8]. TSCs are defined as self-renewing, multipotent cells that give rise to all trophoblast cell lineages (ST, CT, and EVT) that form the fetal part of the developing placenta. In contrast to animal models, human TSCs are quite elusive. Their identification and isolation are much more elaborate, as they are highly restricted to the blastocysts obtained during the *in vitro* fertilization and first-trimester placental tissues [8]. The establishment of efficient TSCs culture requirements may enable creation of novel cell models that could be used as a readily available tool to explain the pathophysiology of the numerous pregnancy complications and as a safe *in vitro* environment for the testing of new drug efficacy.

Human embryonic development

Embryogenesis is initiated by the fusion of oocyte and sperm, which creates a zygote that subsequently divides, achieving the morula stage [9]. Then the blastocyst forms on approximately 4-5th day post-fertilization and consists of inner cell mass (ICM), which give rise to all three germ layers that afterward create embryo and trophectoderm (TE), which is the precursor of trophoblast cells. The endometrium is receptive, allowing embryo implantation for only about 3-5 days of the menstrual cycle [10]. The first stage of human placenta development occurs after implantation that takes place 6-7 days post-conception when polar TE, the part of TE that is contiguous with ICM, attaches to the mucosa inside the uterine cavity [9,11,12]. After implantation, TE divides into two lineages: mononuclear cytotrophoblast and multinucleated syncytium [12,13]. In the following days, primitive syncytium penetrates the maternal endometrium, which is known as decidua [14]. This leads to the creation of fluid-filled lacunar spaces that shortly merge into blood sinusoids as the maternal capillaries are damaged [11,12]. Simultaneously cytotrophoblast starts to invade syncytium forming the first villous-like structures. Subsequently, secondary villi are formed as the core is penetrated by extraembryonic mesenchymal cells. Around 17-18th day post-fertilization, tertiary villi develop as the first fetal blood vessels appear. About the day 32nd post-fertilization, fetal circulation connects with the vessels formed inside the placenta via the umbilical cord. [12–15].

At this time, all types of trophoblast cells, such as syncytiotrophoblast, villous cytotrophoblast, and extravillous trophoblast, are present [13,16]. First-trimester CT cells may differentiate and give rise to other trophoblast cells - ST and EVT [8,16]. Okae et al. suggest that preimplantation blastocyst-derived TE cells and postimplantation CT cells are the sources of human TSCs [8]. Research in mice shows that the homeobox protein CDX-2 (CDX2) is essential to TE differentiation. Expression of CDX2 was also observed in human placenta development. However, it cannot be considered a specific marker of human TSCs because human placenta formation varies from any other species [8,13,16].

ST is covered with microvilli that increase its surface area, which is directly contacted with maternal decidua, participating in gases and nutrients exchange between the fetus and the mother. ST has a crucial role in impeding pathogens penetration into the uterus and partakes in transferring mater-

nal IgG antibodies to the embryo. It also secretes hormones (e.g., human chorionic gonadotropin, somatotropin, or steroid hormones) which modulate physiology and adapt the woman's metabolism to the changes that occur during pregnancy [11,17]. EVT cells have a vital role in the process of anchoring villi to the decidua by transforming the spiral arteries that supply blood to the uterine endometrium. EVT differentiate into interstitial EVT (iEVT), which invades decidual stroma, and endovascular trophoblast EVT (eEVT), which takes part in remodeling of the uterine vessels [11,18].

Trophoblast consists of cells of other derivation either. The stromal part of the villi also comprises connective tissue, blood vessels, and the immune cells. It is believed that these tissues originate from extraembryonic mesenchyme [11].

Apicella et al. indicated the validity of epigenetic mechanisms such as DNA methylation or histone modifications in the proper placenta development [19]. The complexity of DNA expression regulations accompanying human trophoblast differentiation should be taken under consideration in looking for causes of uteroplacental insufficiency, which still till this day is a mystery for modern medicine [20,21].

The role of the placenta in human fetal development

After 10-12 weeks after the fertilization placenta develops the structures called terminal villi. The placental membrane maintains the continuous exchange of gases, nutrients, and waste products. Fetus by diffusion gets oxygen from the maternal blood and gives back carbon dioxide [1,2]. Low blood flow can disturb this process, which could induce the development of various pregnancy-related complications [3]. Regarding the fact that fetal gluconeogenesis is ineffective, the maternal circulation provides the constant glucose supply to the fetus via the protein-mediated facilitated diffusion, which is controlled by the specific glucose transporters protein family (GLUT). The placenta produces numerous hormones, such as progesterone, chorionic gonadotropin, or the placental growth hormone. The placenta's endocrine activity plays one of the most prominent roles in proper fetal development regulation. Pinocytosis of the maternal antibodies, mainly of immunoglobulins G class, protect the fetus during pregnancy and even a few months after birth [22]. The disturbances of the processes mentioned above may lead to various pregnancy complications.

Intrauterine growth restriction (IUGR) is one of the most severe gestational complications. The pathogenesis of that condition is not clear but is usually associated with placental insufficiency. The IUGR increases the risk of stillbirth, multiple congenital malformations, and even the incidence of type 2 diabetes and cardiovascular diseases in adulthood [12,23]. The results of a porcine study indicate that IUGR-MSCs show increased adipogenic and fibrogenic capacity. Their osteogenic and chondrogenic properties were significantly decreased compared to normal MSCs. The central hypothesis that comes from that study about the PD-MSCs' potential role in the pathophysiology of that condition is quite surprising and should be tested in humans [23].

The role of human placenta-derived mesenchymal stem cells is not well-studied in pathological pregnancies. Nonetheless, it is known that these cells can secret many molecules, such as growth factors, cytokines, chemokines, and extracellular vesicles (EVs) [24,25]. We can distinguish amniotic mesenchymal stromal cells and chorionic mesenchymal stromal cells, but their secretory functions are nearly the same [26]. Because the collection and isolation of PD-MSCs, during the pregnancy are somewhat challenging and raise ethical issues, we cannot assess their role in the pregnancy-related pathologies in prospective clinical trials.

Human placental stem cells – characteristic, methods of isolation, molecular research, and potential clinical applications

The broad definition of placenta-derived stem cells includes several types of stem cells that originate from different parts of the human placenta and amniotic membranes. The participants of the "First International Workshop on Placenta-derived Stem Cells," which was held in Brescia, Italy, in 2007, reached a consensus on the nomenclature of the placenta-derived stem cells. They concluded that there are two stem cell populations that derive from the amniotic membranes (human amniotic epithelial cells (hAEC) and human amniotic mesenchymal stromal cells (hAMSC), as well as two subgroups that derive from placenta per se, the population of human chorionic mesenchymal stromal cells (hCMSC) and human chorionic trophoblastic cells (hCTC) [27]. This review focuses mostly on the populations of the mesenchymal and trophoblast stem cell lineages.

Placenta-derived mesenchymal stem cells

The same workshop experts established the hAMSC and hCMSC defining criteria that included the confirmation of their fetal origin, adherence to standard plastic flasks, a capability to form the fibroblast colony-forming units and differentiate into one or more cell lines form the following list – osteogenic, adipogenic, chondrogenic, and vascular/endothelial. Moreover, both stem cell populations have to exhibit the pronounced expression of the following antigens – CD90, CD73, CD105, and do not manifest the expression of the hematopoietic markers - CD45, CD34, CD14, and HLA-DR [27].

It has been established that cell populations classified as hCMSC could be effectively isolated from the various regions of term placentas such as cho-

rionic plate and villous chorion [24]. Samples could be obtained from either the patients who underwent the cesarean section procedure or delivered vaginally [24,28,29]. The fragments of chorionic tissue are surgically dissected from the placentas. After the collection, the placental tissue samples should be soaked in a mixture of cold phosphate-buffered saline (PBS) and antibiotics. The specimens should be transferred to the laboratory immediately after the collection, where the samples are repeatedly washed in PBS, manually separated from the amniotic membranes, and cut into pieces smaller than 1mm. The above-mentioned procedures are usually similar in various isolation protocols. There are numerous possible approaches for further cell preparations. We will shortly describe one of them. Afterward, the prepared material is suspended for 60 minutes in the culture media mixed with the digestive enzymes - collagenase and dispase. Then, the suspension is centrifuged, and the supernatant is discarded. In the next step, the cell pellet is lysed in the ammonium-chloride-potassium (ACK) buffer for 5 minutes and once again centrifuged to separate the pellet from the supernatant. The collected pellet is suspended in the serum-free Dulbecco's modified Eagle's medium (DMEM) or the minimum essential medium Eagle – alpha modification (αMEM) with antibiotics and cultured in the culture flasks in the stable, controlled conditions at 37°C in 5% CO_2/95% air [24,30,31]. Cells adhere to the plastic plates after about three days of cultivation. Cells could be harvested when the cultures reach about 80% confluence or passaged to new culture flasks with fresh growth media [24]. Similarly, to hCMSC, the hAMSC can be isolated from the amniotic membranes' mesenchymal layer [6]. The amnion is manually separated from the chorion, minced into small fragments, and digested by dispase. The fragments are suspended in RPMI complete culture media and treated with collagenase and DNase I. After the centrifugation, the cells are finally suspended and cultured in the Chang medium C with the glutamine at 37°C in 5% CO_2 [28].

It was established that PD-MSCs and MSCs derived from the other tissues possess similar growth properties in the *in vitro* cultures [31]. Nonetheless, the same authors noted that the relative expression of the several stemness marker genes was the lowest in the population of PD-MSCs in comparison to those measured in MSCs derived from the bone marrow, umbilical cord blood, and the adipose tissue. Only the expression of the NANOG, Krüppel-like factor 4 (KLF4), and Inhibin beta A (INHBA) was detected in the PD-MSCs population. They did not detect the expression of the octamer-binding transcription factor 4 (OCT4), sex-determining region Y-box 2 (SOX2), MYC, LIN28, and REX1 genes [31]. Heo et al. reported that PD-MSCs had the potential to differentiate into adipocytes and chondrocytes but did not acquire specific features of osteogenic differentiation [31]. Some authors examined the osteogenic potential of PD-MSCs obtained from various parts of the placenta. They reported that stem cells isolated from the amniotic membranes have higher osteogenic differentiation potency than the chorionic ones [4,5]. In contrast to those results, the hCMSC had a higher potential to differentiate into the adipocytes [4].

Multiple studies revealed that PD-MSCs themselves produce several anti-inflammatory cytokines and excrete the nanovesicles, called the exosomes, which may possess extraordinary regenerative properties [32]. The collection and isolation of the PD-MSCs' exosomes are quite complex, multi-stage procedures. Briefly, in normal conditions, the exosomes are released continuously out of the cells into the culture media. After 48 hours of incubation, the conditioned culture media is collected and sequentially centrifuged following the previously described isolation and purification protocols [30,33–35]. The isolated PD-MSCs' exosomes are identified by the membrane presence of CD63 antigen [30]. It was reported that the treatment of myoblasts with the purified PD-MSCs' exosomes or conditioned media increased their differentiation and decreased the expression of fibrogenic genes. Moreover, PD-MSCs transplantation significantly reduced the level of inflammation and fibrosis in mdx mice – a mouse model for studying Duchenne muscular dystrophy. It was speculated that those activities could be evoked by the mi-RNA molecules [32]. Furthermore, conditioned medium from the PD-MSCs and purified PD-MSCs' exosomes have an ability to stimulate the secretion of several proangiogenic cytokines, and as a result, to increase the viability and support the migration of the endothelial cells as well as enhance endothelial tube formation *in vitro* [24,36].

There have been few studies that analyzed the properties of these cells in the treatment of common human diseases. Most studies are based on the use of animal models. Abumaree et al. showed that PD-MSCs have an impact on lymphocytes. To be exact, they have immunosuppressive properties, which could be used in the autoimmune diseases' treatment protocols [37]. PD-MSCs also reduce the blood concentrations of interleukin-5, which could have a significant impact on the treatment of asthma [29]. Other studies proved that PD-MSCs and their exosomes demonstrate proangiogenic activity so that they might be a new treatment method for ischemic diseases or dermal wounds [24,38–40]. Moreover, PD-MSCs with NK4 molecule expression have antitumor activity, and they are ideal vectors to target the glioblastoma multiforme, which is the most common CNS-tumor with an abysmal prognosis [41]. There are several reports that PD-MSCs could also be used to cure neurological diseases like

Duchenne muscular dystrophy, multiple sclerosis, spina bifida, or autoimmune encephalomyelitis. Their EVs contain proteins, which provide neuronal survival and development. That promotes myelin regeneration by inducing oligodendrocyte precursor cells to differentiate into mature myelinating oligodendrocytes [25,32,35,42,43]. Furthermore, dementias reportedly can be treated with PD-MSCs because of their convalescent effect on cognitive behavior, connected with the recovery of the cholinergic neuronal populations [44]. Park et al. used murine PD-MSCs to treat diabetic gastropathy in mice, giving positive results [45]. All these studies show that PD-MSCs have immense potential in almost every field of medicine.

Trophoblast stem cells

Numerous animal studies focused on TSCs isolation and their other cellular characteristic [46–50]. Human studies are less accessible mainly due to the possible ethical dilemmas that may arise when the laboratory procedures are performed on the human tissues obtained during early pregnancy. After the years of ongoing investigations, it has been announced that the human trophoblast stem cells can be efficiently directly isolated from the blastocysts-stage embryos and first-trimester placental tissues, obtained from the planned terminations, and then long-term cultured under the strictly regulated conditions [8]. The optimal culture medium should include CHIR99021 (a Wingless/Integrated (WNT) activator), epidermal growth factor (EGF), A83-01 and SB431542 (transforming growth factor-beta inhibitors), valproic acid (a histone deacetylase inhibitor), and Y27632 (a Rho-associated protein kinase inhibitor) [8]. More studies analyzed the population of TSCs that derived from the induced pluripotent stem cells (iPSCs) [51–54]. However, TSCs isolated from the fetal tissues are treated as a gold standard in analyzing their biological properties. TSCs *in vitro* cultures appear to be a promising model for testing novel drugs' safety and efficacy for pregnancy-related complications, such as preeclampsia or gestational diabetes. Regarding their limited plasticity, it is unlikely that TSCs could be implemented in the regenerative medicine cell treatment protocols. However, the influence of their secretory profile on the other somatic cell lineages has not been studied yet.

Conclusions

Placenta-derived stem cells definition includes a heterogeneous group of stem cells with various plasticity and self-renewal capacities. Mesenchymal stem cells could be relatively easily isolated from term placentas and amniotic membranes. Their isolation does not raise ethical issues because those tissues are treated as medical waste after delivery, making them readily available. Moreover, PD-MSCs have well described and optimized culture requirements. Both mesenchymal cell lines could be applied in future cell treatment protocols. Molecular analyses revealed that their regenerative properties are connected with their paracrine activity – secretion of numerous cytokines and exosomes. Even though the first ground-breaking reports on the successful outcomes of the human trophoblast stem cells isolation and culture were published, those cells remain elusive and do not cease to be intriguing.

Ethical approval

The conducted research is not related to either human or animal use.

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Conflict of interest statement:

The authors declare they have no conflict of interest.

References:

1. Huppertz B. The anatomy of the normal placenta. J Clin Pathol. 2008;61:1296–302; DOI:10.1136/jcp.2008.055277.
2. Aplin JD, Myers JE, Timms K, Westwood M. Tracking placental development in health and disease. Nat Rev Endocrinol. 2020;16:479–94; DOI:10.1038/s41574-020-0372-6.
3. Huppertz B. Placental origins of preeclampsia: Challenging the current hypothesis. Hypertension. 2008;51; DOI:10.1161/HYPERTENSIONAHA.107.107607.
4. Ma J, Wu J, Han L, Jiang X, Yan L, Hao J, Wang H. Comparative analysis of mesenchymal stem cells derived from amniotic membrane, umbilical cord, and chorionic plate under serum-free condition. Stem Cell Res Ther. 2019;10; DOI:10.1186/s13287-018-1104-x.
5. Shen C, Yang C, Xu S, Zhao H. Comparison of osteogenic differentiation capacity in mesenchymal stem cells derived from human amniotic membrane (AM), umbilical cord (UC), chorionic membrane (CM), and decidua (DC). Cell Biosci. 2019;9; DOI:10.1186/s13578-019-0281-3.
6. Magatti M, Pianta S, Silini A, Parolini O. Isolation, culture, and phenotypic characterization of mesenchymal stromal cells from the amniotic membrane of the human term placenta. Methods Mol. Biol., 2016; DOI:10.1007/978-1-4939-3584-0_13.
7. Murphy MB, Moncivais K, Caplan AI. Mesenchymal stem cells: Environmentally responsive therapeutics for regenerative medicine. Exp Mol Med. 2013;45; DOI:10.1038/emm.2013.94.
8. Okae H, Toh H, Sato T, Hiura H, Takahashi S, Shirane K, Kabayama Y, Suyama M, Sasaki H, Arima T. Derivation of Human Trophoblast Stem Cells. Cell Stem Cell. 2018;22; DOI:10.1016/j.stem.2017.11.004.
9. Hemberger M, Hanna CW, Dean W. Mechanisms of early placental development in mouse and humans. Nat Rev Genet. 2020;21:27–43; DOI:10.1038/s41576-019-0169-4.
10. Ashary N, Tiwari A, Modi D. Embryo Implantation: War in times of love. Endocrinology. 2018;159:1188–98; DOI:10.1210/en.2017-03082.
11. Turco MY, Moffett A. Development of the human placenta. Dev. 2019;146:1–14; DOI:10.1242/dev.163428.
12. Knöfler M, Haider S, Saleh L, Pollheimer J, Gamage TKJB, James J. Human placenta and trophoblast development: key molecular mechanisms and model systems. Cell Mol Life Sci. 2019;76:3479–96; DOI:10.1007/s00018-019-03104-6.
13. Gamage TKJB, Chamley LW, James JL. Stem cell insights into human trophoblast lineage differentiation. Hum Reprod Update. 2016;23:77–103; DOI:10.1093/humupd/dmw026.

14. Boss AL, Chamley LW, James JL. Placental formation in early pregnancy: How is the centre of the placenta made? Hum Reprod Update. 2018;24:750–60; DOI:10.1093/humupd/dmy030.
15. Chang CW, Parast MM. Human trophoblast stem cells: Real or not real? Placenta. 2017;60; DOI:10.1016/j.placenta.2017.01.003.
16. Tiruthani K, Sarkar P, Rao B. Trophoblast differentiation of human embryonic stem cells. Biotechnol J. 2013;8:421–33; DOI:10.1002/biot.201200203.
17. Staud F, Karahoda R. Trophoblast: The central unit of fetal growth, protection and programming. Int J Biochem Cell Biol. 2018;105:35–40; DOI:10.1016/j.biocel.2018.09.016.
18. Turco MY, Gardner L, Kay RG, Hamilton RS, Prater M, Hollinshead MS, McWhinnie A, Esposito L, Fernando R, Skelton H, Reimann F, Gribble FM, Sharkey A, Marsh SGE, O'rahilly S, Hemberger M, Burton GJ, Moffett A. Trophoblast organoids as a model for maternal–fetal interactions during human placentation. Nature. 2018;564:263–81; DOI:10.1038/s41586-018-0753-3.
19. Apicella C, Ruano CSM, Méhats C, Miralles F, Vaiman D. The role of epigenetics in placental development and the etiology of preeclampsia. Int J Mol Sci. 2019;20; DOI:10.3390/ijms20112837.
20. Gamage TKJB, Schierding W, Hurley D, Tsai P, Ludgate JL, Bhoothpur C, Chamley LW, Weeks RJ, Macaulay EC, James JL. The role of DNA methylation in human trophoblast differentiation. Epigenetics. 2018;13:1154–73; DOI:10.1080/15592294.2018.1549462.
21. Silver RM. Examining the link between placental pathology, growth restriction, and stillbirth. Best Pract Res Clin Obstet Gynaecol. 2018;49:89–102; DOI:10.1016/j.bpobgyn.2018.03.004.
22. Gude NM, Roberts CT, Kalionis B, King RG. Growth and function of the normal human placenta. Thromb Res. 2004;114:397–407; DOI:10.1016/j.thromres.2004.06.038.
23. Weatherall EL, Avilkina V, Cortes-Araya Y, Dan-Jumbo S, Stenhouse C, Donadeu FX, Esteves CL. Differentiation Potential of Mesenchymal Stem/Stromal Cells Is Altered by Intrauterine Growth Restriction. Front Vet Sci. 2020;7:1–9; DOI:10.3389/fvets.2020.558905.
24. Komaki M, Numata Y, Morioka C, Honda I, Tooi M, Yokoyama N, Ayame H, Iwasaki K, Taki A, Oshima N, Morita I. Exosomes of human placenta-derived mesenchymal stem cells stimulate angiogenesis. Stem Cell Res Ther. 2017;8; DOI:10.1186/s13287-017-0660-9.
25. Clark K, Zhang S, Barthe S, Kumar P, Pivetti C, Kreutzberg N, Reed C, Wang Y, Paxton Z, Farmer D, Guo F, Wang A. Placental Mesenchymal Stem Cell-Derived Extracellular Vesicles Promote Myelin Regeneration in an Animal Model of Multiple Sclerosis. Cells. 2019;8; DOI:10.3390/cells8121497.
26. Antoniadou E, David AL. Placental stem cells. Best Pract Res Clin Obstet Gynaecol. 2016;31:13–29; DOI:10.1016/j.bpobgyn.2015.08.014.
27. Parolini O, Alviano F, Bagnara GP, Bilic G, Bühring H-J, Evangelista M, Hennerbichler S, Liu B, Magatti M, Mao N, Miki T, Marongiu F, Nakajima H, Nikaido T, Portmann-Lanz CB, Sankar V, Soncini M, Stadler G, Surbek D, Takahashi TA, Redl H, Sakuragawa N, Wolbank S, Zeisberger S, Zisch A, Strom SC. Concise Review: Isolation and Characterization of Cells from Human Term Placenta: Outcome of the First International Workshop on Placenta Derived Stem Cells. Stem Cells. 2008;26; DOI:10.1634/stemcells.2007-0594.
28. Papait A, Vertua E, Magatti M, Ceccariglia S, De Munari S, Silini AR, Sheleg M, Ofir R, Parolini O. Mesenchymal Stromal Cells from Fetal and Maternal Placenta Possess Key Similarities and Differences: Potential Implications for Their Applications in Regenerative Medicine. Cells. 2020;9; DOI:10.3390/cells9010127.
29. Lin SC, Liou YM, Ling TY, Chuang YH, Chiang BL. Placenta-derived mesenchymal stem cells reduce the interleukin-5 level experimentally in children with asthma. Int J Med Sci. 2019;16; DOI:10.7150/ijms.33590.
30. Tooi M, Komaki M, Morioka C, Honda I, Iwasaki K, Yokoyama N, Ayame H, Izumi Y, Morita I. Placenta Mesenchymal Stem Cell Derived Exosomes Confer Plasticity on Fibroblasts. J Cell Biochem. 2016;117; DOI:10.1002/jcb.25459.
31. Heo JS, Choi Y, Kim HS, Kim HO. Comparison of molecular profiles of human mesenchymal stem cells derived from bone marrow, umbilical cord blood, placenta and adipose tissue. Int J Mol Med. 2016;37; DOI:10.3892/ijmm.2015.2413.
32. Bier A, Berenstein P, Kronfeld N, Morgoulis D, Ziv-Av A, Goldstein H, Kazimirsky G, Cazacu S, Meir R, Popovtzer R, Dori A, Brodie C. Placenta-derived mesenchymal stromal cells and their exosomes exert therapeutic effects in Duchenne muscular dystrophy. Biomaterials. 2018;174; DOI:10.1016/j.biomaterials.2018.04.055.
33. Théry C, Amigorena S, Raposo G, Clayton A. Isolation and Characterization of Exosomes from Cell Culture Supernatants and Biological Fluids. Curr Protoc Cell Biol. 2006;30; DOI:10.1002/0471143030.cb0322s30.
34. Tschuschke M, Kocherova I, Bryja A, Mozdziak P, Angelova Volponi A, Janowicz K, Sibiak R, Piotrowska-Kempisty H, Iżycki D, Bukowska D, Antosik P, Shibli JA, Dyszkiewicz-Konwińska M, Kempisty B. Inclusion Biogenesis, Methods of Isolation and Clinical Application of Human Cellular Exosomes. J Clin Med. 2020;9:436; DOI:10.3390/jcm9020436.
35. Kumar P, Becker JC, Gao K, Carney RP, Lankford L, Keller BA, Herout K, Lam KS, Farmer DL, Wang A. Neuroprotective effect of placenta-derived mesenchymal stromal cells: role of exosomes. FASEB J. 2019;33; DOI:10.1096/fj.201800972R.
36. König J, Weiss G, Rossi D, Wankhammer K, Reinisch A, Kinzer M, Huppertz B, Pfeiffer D, Parolini O, Lang I. Placental mesenchymal stromal cells derived from blood vessels or avascular tissues: What is the better choice to support endothelial cell function? Stem Cells Dev. 2015;24; DOI:10.1089/scd.2014.0115.
37. Abumaree MH, Abomaray FM, Alshabibi MA, AlAskar AS, Kalionis B. Immunomodulatory properties of human placental mesenchymal stem/stromal cells. Placenta. 2017;59:87–95; DOI:10.1016/j.placenta.2017.04.003.
38. Kamprom W, Kheolamai P, U-Pratya Y, Supokawej A, Wattanapanitch M, Laowtammathron C, Roytrakul S, Issaragrisil S. Endothelial Progenitor Cell Migration-Enhancing Factors in the Secretome of Placental-Derived Mesenchymal Stem Cells. Stem Cells Int. 2016;2016; DOI:10.1155/2016/2514326.
39. Mathew SA, Naik C, Cahill PA, Bhonde RR. Placental mesenchymal stromal cells as an alternative tool for therapeutic angiogenesis. Cell Mol Life Sci. 2020;77:253–65; DOI:10.1007/s00018-019-03268-1.
40. Ertl J, Pichlsberger M, Tuca AC, Wurzer P, Fuchs J, Geyer SH, Maurer-Gesek B, Weninger WJ, Pfeiffer D, Bubalo V, Parvizi D, Kamolz LP, Lang I. Comparative study of regenerative effects of mesenchymal stem cells derived from placental amnion, chorion and umbilical cord on dermal wounds. Placenta. 2018;65:37–46; DOI:10.1016/j.placenta.2018.04.004.
41. Jabbarpour Z, Kiani J, Keshtkar S, Saidijam M, Ghahremani MH, Ahmadbeigi N. Effects of human placenta-derived mesenchymal stem cells with NK4 gene expression on glioblastoma multiforme cell lines. J Cell Biochem. 2020;121:1362–73; DOI:10.1002/jcb.29371.
42. Shende P, Subedi M. Pathophysiology, mechanisms and applications of mesenchymal stem cells for the treatment of spinal cord injury. Biomed Pharmacother. 2017;91:693–706; DOI:10.1016/j.biopha.2017.04.126.
43. Jiang H, Zhang Y, Tian K, Wang B, Han S. Amelioration of experimental autoimmune encephalomyelitis through transplantation of placental derived mesenchymal stem cells. Sci Rep. 2017;7:1–16; DOI:10.1038/srep41837.
44. Cho JS, Lee J, Jeong DU, Kim HW, Chang WS, Moon J, Chang JW. Effect of placenta-derived mesenchymal stem cells in a dementia rat model via microglial mediation: A comparison between stem cell transplant methods. Yonsei Med J. 2018;59:406–15; DOI:10.3349/ymj.2018.59.3.406.
45. Park JM, Lee SH, Kim K Il, Kim WH, Cho JY, Hahm KB, Hong SP. Feasibility of intraperitoneal placental-derived mesenchymal stem cell injection in stomachs of diabetic mice. J Gastroenterol Hepatol. 2018;33:1242–7; DOI:10.1111/jgh.14045.
46. Tanaka S, Kunath T, Hadjantonakis AK, Nagy A, Rossant J. Promotion to trophoblast stem cell proliferation by FGF4. Science (80-). 1998;282; DOI:10.1126/science.282.5396.2072.
47. Kubaczka C, Senner C, Araúzo-Bravo MJ, Sharma N, Kuckenberg P, Becker A, Zimmer A, Brüstle O, Peitz M, Hemberger M, Schorle H. Derivation and maintenance of murine trophoblast stem cells under defined conditions. Stem Cell Reports. 2014;2; DOI:10.1016/j.stemcr.2013.12.013.
48. Rivron NC, Frias-Aldeguer J, Vrij EJ, Boisset JC, Korving J, Vivié J, Truckenmüller RK, Van Oudenaarden A, Van Blitterswijk CA, Geijsen N. Blastocyst-like structures generated solely from stem cells. Nature. 2018;557; DOI:10.1038/s41586-018-0051-0.
49. Adachi K, Nikaido I, Ohta H, Ohtsuka S, Ura H, Kadota M, Wakayama T, Ueda HR, Niwa H. Context-Dependent Wiring of Sox2 Regulatory Networks for Self-Renewal of Embryonic and Trophoblast Stem Cells. Mol Cell. 2013;52; DOI:10.1016/j.molcel.2013.09.002.
50. Ohinata Y, Tsukiyama T. Establishment of trophoblast stem cells under defined culture conditions in mice. PLoS One. 2014;9; DOI:10.1371/journal.pone.0107308.
51. Horii M, Li Y, Wakeland AK, Pizzo DP, Nelson KK, Sabatini K, Laurent LC, Liu Y, Parast MM. Human pluripotent stem cells as a model of trophoblast differentiation in both normal development and disease. Proc Natl Acad Sci U S A. 2016;113; DOI:10.1073/pnas.1604747113.
52. Horii M, Bui T, Touma O, Cho HY, Parast MM. An Improved Two-Step Protocol for Trophoblast Differentiation of Human Pluripotent Stem Cells. Curr Protoc Stem Cell Biol. 2019;50; DOI:10.1002/cpsc.96.
53. Roberts RM, Loh KM, Amita M, Bernardo AS, Adachi K, Alexenko AP, Schust DJ, Schulz LC, Telugu BPVL, Ezashi T, Pedersen RA. Differentiation of trophoblast cells from human embryonic stem cells: To be or not to be? Reproduction. 2014;147; DOI:10.1530/REP-14-0080.
54. Amita M, Adachi K, Alexenko AP, Sinha S, Schust DJ, Schulz LC, Roberts RM, Ezashi T. Complete and unidirectional conversion of human embryonic stem cells to trophoblast by BMP4. Proc Natl Acad Sci U S A. 2013;110; DOI:10.1073/pnas.1303094110.