CELL TECHNOLOGIES IN BIOLOGY AND MEDICINE

Application Potential of Human Fetal Stem/Progenitor Cells in Cell Therapy

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We analyzed the possibility of using fetal stem and progenitor cells for the treatment of various pathologies. A comparative characteristic of stem cells from fetuses and adult donors is presented and advantages of the use of fetal biometerial for biotransplantation are described. The main tissue sources of fetal stem cells are characterized and experimental and clinical data on the use of fetal cells for cytotherapy are presented.

Key Words: *cell therapy; fetal stem cells*

Cell technologies are now finding ever-widening application in clinical practice. Recent progress in these technologies is determined by latest achievements of cell and molecular biology in the studies of stem cells (SC) and their early descendants. New methods for obtaining enriched populations of SC and progenitor cells in *in vitro* culture, their targeted differentiation, and genetic modification of SC were developed.

However, the choice of the source of donor cells is probably the key problem for successful cell therapy. Autologous and allogeneic SC from adult donors are most widely used for transplantation to recipients. The pool of SC and their differentiation potential decrease during postnatal ontogeny.

Fetal cells can be an alternative source of donor biomaterial, but the use of these cells is restricted due to moral and ethical problems and legal acts of some countries. Clinical use of umbilical cord blood and umbilical cord cells of newborns and amniotic fluid cells can help to solve these problems.

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We discussed different aspects of application of cell therapy with the use of various types of transplanted cells, determined conditions for successful experimental and clinical application of cell technologies, and some limitations for the use of donor biomaterial obtained from different sources.

Fetal SC as optimal cell source for cytotherapy

Multipotent fetal SC possess higher differentiation potential than SC of adults and hence, they can generate a wider spectrum of progenitor cells. In contrast to embryonic SC (ESC), fetal SC do not form teratomas after transplantation to the recipient. In recipient organism, these cells undergo terminal differentiation in the appropriate tissue microenvironment and/or in accordance with their intrinsic genetic program. They can grow, migrate, and form functional contacts with recipient cells. Fetal SC produce great amounts of angiogenic, neurotrophic, and other factors, which promote engrafting of the transplant and regeneration of the surrounding tissues. Fetal tissues, especially at early terms of gestation, are a rich source of progenitor cells, early SC descendants, which can be used in cell therapy. They are characterized by high pro-

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liferative potential and are committed to a certain differentiation lineage. Hence, progenitor cells are involved into regeneration process in the corresponding tissue environment at earlier terms than SC. Oxygen demands of fetal cells are considerably lower than of cells in mature tissues, therefore they are more resistant to ischemic damage during isolation, *in vitro* culturing, and after transplantation. Due to the absence of close cell-cell contacts, fetal cells are more resistant to traumatic injuries during isolation, are easily transferred into suspension culture, and retain high viability during transportation. This property also contributes to high integrity of fetal cells during cryopreservation [12].

Fetal SC are ideal targets for genetic modification because of high proliferative activity and more intensive expression of amphotropic receptors on their surface compared to SC from adult bone marrow. Intensive proliferation of fetal SC facilitates clonal analysis of transfected cells. The efficiency of transduction of fetal SC is higher compared to transduction of the vector into SC from adult bone marrow [7].

From immunological point of view, fetal cells are more preferable for allotransplantation than cells from adult donors.

Histocompatibility antigens are less intensively expressed on fetal cells of early gestation terms than on adult cells, which determines their higher resistance to rejection. Fetal SC obtained from fetuses before 12 weeks gestation are not rejected by the immune system of the recipient [27]. After intravenous injection of fetal liver cells from mice of 13-15 gestation days to allogeneic animals, the transplanted cells are distributed into various organs of the recipient and after 60 days their content in tissues is about 2.75%. No inflammatory reaction to injection of allogeneic fetal cells was observed [10]. Similarly, xenotransplants of neural SC (NSC) of human fetuses are not rejected in rats with spinal trauma in the absence of immunosuppressive therapy. Human fetal NSC locally injected into rat spinal cord at the site of traumatic injury retain viability for at least 3 months [33]. Thus, fetal SC and their descendants isolated from the corresponding tissues can be successfully used for the treatment of various diseases and are involved into the processes of tissue regeneration.

However, cell technologies based on the use of SC and their early descendants can be introduced into clinical practice only if their safety, primarily, oncogenic safety, is guaranted. Potential risk of oncogenic transformation of SC during *in vitro* culturing and after transplantation is evident from the fact that they express common markers with ESC and many malignized cell lines. Transformed cells appeared in a population of human multipotent MSC isolated from subcutaneous fat after 2-month *in vitro* culturing (more than 20 divisions). After transplantation of these cells to immunodeficient SCID mice, tumors developed in 100% recipients. Spontaneous transformation is not a unique phenomenon, it was observed in all samples of passaged MSC [21]. According to other authorities, multiple passages of SC *in vitro* do not lead to their oncogenic transformation. It is obvious that oncogenic transformation of SC can be induced by isolation and *in vitro* culturing conditions. Karyotyping of cells intended for transplantation is a strictly obligatory procedure.

Another potential risk, fusion of donor cells with recipient cells (formation of heterokatyons), should also be taken into account in the therapy with SC and their early descendants. The possibility of this scenario was described in some experimental studies. It was demonstrated that human and murine bone marrow cells after their transplantation can spontaneously fuse with hepatocytes, skeletal myocytes, cardiomyocytes, and brain cells of the recipient. The formation of heterokaryons can led to unwanted consequences. Fusion of bone marrow cells with nerve cells in spinal ganglia and sciatic nerve during cell therapy of experimental diabetes mellitus in rats impaired neuronal function and induced their preamture apoptosis [34]. Unfortunately, the possibility and posttransplantation consequences of the formation of donor/recipient cytohybrids can not be evaluated on the basis of the results obtained by other investigators.

Tissue sources of fetal SC and their progenitors

Fetal liver. Human fetal liver cells are used as an alternative source of hemopoietic SC (HSC) for *in vivo* hemopoiesis recovery [31]. Despite low content of HSC in fetal liver, sufficient amounts of cells for transplantation can be obtained during *in vitro* culturing. Proliferative and differentiation potential of HSC decreases during ontogeny. The efficiency of engrafting of HSC from fetal liver depends on their dose and transplantation route. The presence of alloreactive T lymphocytes should be taken into account. These cells appear in the fetal liver starting from 13 week gestation. In immunodeficient recipient fetuses, HSC from fetal liver have a 10-fold competitive advantage compared to SC from adult bone marrow.

First trimester fetal liver is also a source of MSC. Fetal MSC and their analogues in adults are mor-

phologically similar, but differ phenotypically and are superior to adult MSC by proliferative and differentiation potential and immunosuppressor activity. For instance, fetal MSC express pluripotency markers Oct-4, Nanog, Rex-1, SSEA-3, SSEA-4, Tra-1-60, and Tra-1-81. Fetal MSC are characterized by long telomeres, high telomerase activity, and intensive expression of hTERT. They more effectively proliferate and later age in culture than their analogues in adults [16].

Serial analysis of gene expression in MSC from fetal liver during trimester I and adult bone marrow revealed 70 genes that are more intensively expressed in fetal liver MSC compared to the analogous cells in adults. These are transcripts of proteins regulating germ plasm and limb patterning, brain and early muscle development. Transcripts implicated in cell cycle promotion, chromatin regulation and DNA repair were also more abundant in fetal MSC. At the same time, expression of genes responsible for differentiation of smooth muscle cells, keratinocytes, and immune functions is decreased. Fetal MSC are characterized by more intensive expression of ICAM-1 and HLA-G and lower level of expression of class I and II HLA molecules and VCAM-1 compared to MSC of adults. Thus, fetal MSC have higher proliferative capacity and are less lineage committed than adult MSC [15].

Hepatocyte precursors expressing hemopoietic markers CD34 and CD177 are isolated from the liver of the first- and second-trimester fetuses. During trimester I, cells simultaneously carrying hemopoietic and pancreatic markers were identified, whereas cells with hepatocyte markers appear during trimester II. A new class of multipotent precursors with immunophenotype $CD34^+$, $CD90^+$, $c\text{-}kit^+$, $EPCAM^+$, $c\text{-}met^+$, $SSEA\text{-}4^+$, $CD44h^+$, expressing also cytokeratins (CK18 and CK19) and vimentin was isolated from fetal liver (gestation days 74-108). During passages 1-10, the cells are identical by morphology, immunophenotype, length of telomeres, and differentiation capacities. In the appropriate induction medium, they differentiate into hepatocytes and bile duct cells, as well as into the bone, adipose, cartilaginous tissues, and endothelium. It is assumed that this type of SC is presented by mesodermal cells at the stage of mesenchymal-epithelial transition. After transplantation to animals with experimental damage to the liver, the cells survive and differentiate into functional hepatocytes [9].

Bone marrow. The content of $CD34^+$ HSC in the fetal bone marrow is higher than in the adult bone marrow (2-10% *vs.* 1%). Similarly to fetal liver, bone marrow contains a population of MSC (1/10,000 cells during trimester II). The content of MSC in the bone marrow decreases with age (adult bone marrow contains 1 MSC pre 250,000 cells).

By the clonogenic potential, $CD34^+$ cells of fetal bone marrow considerably surpass HSC from the umbilical cord blood and from the adult bone marrow and peripheral blood. In mixed lymphocyte culture, the proliferative response of T cells to HSC from the fetal bone marrow and umbilical cord blood is considerably lower than to adult $CD34^+$ cells. Analysis of cytogenetic profile showed that the percent of S-phase cells among SC from fetal bone marrow and umbilical cord blood cells is one order of magnitude higher than in the population of HSC in adults. Stromal cells of fetal bone marrow are characterized by higher expression of cytokines, granulocyte-macrophage CSF, granulocytic CSF, macrophage CSF, IL-6, and IL-11 compared to similar cells from other sources. These cytokines are essential for engrafting and homing of transplanted SC. Thus, fetal bone marrow is the best source of SC for cell therapy due to exclusively high proliferative activity, low immunogenicity, and high content of primitive SC/progenitor cells. It should be emphasized that SC from fetal bone marrow (16-20 week gestation) are in an optimum regimen of hemopoiesis [26].

Fetal nerve tissue. Culturing of cells isolated from human fetal brain tissue at early terms of gestation (weeks 14-19) in the presence of EGF and FGF led to the formation of neurospheres, spherical cell aggregates containing nestin-positive NSC and vimentin-positive progenitor cells. NSC *in vitro* and *in vivo* give rise to three cell types: neurons, astrocytes, and oligodendrocytes.

A NSC cell line was obtained from human fetal cortical tissue by V-myc immortalization. These cells are capable of self-maintenance for more than 50 passages. In the presence of EGF and FGF they express nestin, vimentin, and Sox2. After removal of growth factors from the culture medium, proliferation of fetal NSC is accompanied by a decrease in V-myc and telomerase expression, they differentiate into glial cells and neurons (primarily glutamatergic and GABA-ergic, as well as into hydrolase-positive, presumably dopaminergic neurons). Expression of genes associated with regionalization and cell commitment (Pax6, Emx2, and neurogenin-2) in cortical precursors during brain development is maintained [6].

Additional sources of fetal SC. Fetal lungs, pancreas, and kidneys are also the sources of MSC. During the second trimester, fetal lungs contain maximum number of MSC. After 6-week culturing *in vitro*, the yield of these cells was 1.4×10^3. During the first trimester, MSC of pulmonary origin do not

express CD34, and during the second trimester they acquire this marker, but do not express CD45, which attests to their non-hemopoietic origin. The percent of $CD34^+CD45^-$ cells in the lungs was higher (44%) than in fetal bone marrow (4.8%), spleen (12.6%), or liver (7.5%) at this term of gestation. During *in vitro* culturing, MSC from fetal lungs lose endothelial marker CD34 [19].

In the pancreas, MSC appear between the 4th and 6th months of gestation. Under the effect of specific inductors, 80% these cells undergo chondrogenic, osteogenic, or adipogenic differentiation.

Nestin-positive precursor cells present in the fetal pancreas can differentiate into endocrine cells of the pancreas. SC isolated from the ducts of the pancreas can also differentiate into insulin-producing cells *in vitro* [18].

Fetal kidneys represent another source of epithelial and stromal cell lines. Metanephric mesenchymal cells do not express CD45, CD34, and other hemopoietic markers, but carry mesenchymal markers vimentin, laminin, and type 1 collagen. Under *in vitro* conditions, these cells acquire osteogenic or myogenic phenotype [4].

Amniotic fluid. At the early terms of pregnancy, amniotic fluid contains cells of the amniotic membrane, while fetal cells, derivatives of the urinary, respiratory, and gastrointestinal tracts appear starting from the second trimester. The second-trimester amniotic fluid contains classical MSC analogical by the immunophenotypical profile to bone marrow MSC. They express CD29, CD44, CD73, CD105, and CD166. These cells undergo osteogenic, adipogenic, chondrogenic, and myogenic differentiation *in vitro*. In matrigel MSC spontaneously differentiate *in vitro* into endotheocytes. Expression of specific endothelial markers FLT-1, KDR, ICAM-1 in differentiating endotheliocytes increases in the presence of vascular endothelial growth factor (VEGF). MSC expressing transcription factors and genes of cardiomyocytes were also detected among these cells. They can differentiate into cardiomyocyte-like cells *in vitro* [11].

Umbilical cord blood. Umbilical cord blood contains greater number of precursor cells compared to peripheral blood of adults. Apart from $CD34^+$ HSC, umbilical cord blood contains $CD133^+$ HSC, earlier hemopoietic precursors with very high hemopoietic potential. The population of HSC includes self-maintaining HSC and progenitor cells giving rise to functionally active specialized cells of the blood and immune system. Progenitor cells play an important role in reconstitution of the immune function in recipients at the early terms after myeloablative therapy. HSC ensure the hemopoietic function at later terms. Umbilical cord blood also contains classical MSC [33].

Extraembryonic tissues

Placenta. MSC constitute less than 1% placental cells. They proliferate with the same intensity as mesenchymal amniocytes. Similarly to mesenchymal amniocytes, but in contrast to mature MSC, placental cells during trimester I and after delivery express cytokeratins CK-8 and CK-18. They also produce hemopoietic cytokines (IL-6, CSF-1, and ligand Flt-3). Placental MSC undergo adipogenic and osteogenic differentiation and exhibit immunoreguilatory properties [37].

The placenta also contains multipotent cells with higher immunosuppressor potential compared to bone marrow MSC. They express indolamine-2,3-dioxygenase and intracellular HLA-G. These cells suppress alloantigen- and mitogens-induced proliferation of $CD4^+$ and $CD8^+$ T lymphocytes. Their immunosuppressor effect decreases in the presence of antibodies to anti-inflammatory cytokines IL-10 and TGF-β.

During pregnancy, a reciprocal transplacental exchange of cells and nucleic acids is observed between the mother and fetus. It is hypothesized that transferred cells participate in the mechanisms of tissue reparation in different organs of the mother and fetus [5]. Many SC and their early descendants are present among fetal cells crossing the placental barrier. Maternal cells also enter the fetal circulation and can persist in the fetus even until adulthood (microchimerism phenomenon). Fetal cells transferred from to the mother can migrate during pregnancy into various tissue compartments, in particular into the liver and skin. They can cross the blood-brain barrier, penetrate into injured brain areas, and participate in reparation of the nervous tissue by generating the precursors of mature cells [5,22]. A small population of functionally active fetal cells with features typical of SC cells (so-called pregnancy-associated progenitor cells) probably persists in the circulation and tissue after delivery; they most likely play a protective role and participate in tissue reparation [22]. This reflects high plasticity and migration potential of fetal SC, which is the main indication for their use in cell therapy. Evaluation of structural characteristics of fetal SC responsible for their capacity to cross the blood-brain barrier will make it possible to use fetal NSC and progenitor cells for the therapy of various pathologies of the central nervous system.

Thus, fetal SC can be isolated not only from the blood and hemopoietic organs of fetuses at early

terms of gestation, but also from somatic organs, amniotic fluid, and placenta throughout the pregnancy. Fetal blood is a rich source of HSC that proliferate more intensively than SC of the umbilical cord blood and adult bone marrow. The blood, liver, and bone marrow of the first-trimester fetuses also contain a population of MSC that are more primitive and possess wider differentiation potential than their analogues in adults. Fetal SC is an intermediate cell type between ESC and postnatal SC and therefore are more preferable source of donor material for cell therapy.

Fetal cell therapy

Neurological diseases. *In vivo* studies on experimental animals showed that implantation of NSC or their progenitors into the brain can be promising for the therapy of neurological diseases such as stroke, some neurodegenerative diseases (Huntington, Parkinson, and Alzheimer diseases), and traumatic injuries of the spinal cord.

Stable immortalized NSC lines were obtained in primary cultures of human fetal telencephalon using a retroviral vector V-myc. NSC line HB1.F3 has normal karyotype, expresses nestin, constantly proliferates, and can differentiate into neuronal and glial cells. This NSC line was used for the treatment of mice with experimental hemorrhagic stroke. Transplanted NSC infiltrate perihematoma zones and differentiate into neurons and astrocytes. After 2-8 weeks, the recovery of brain functions in mice with NSC xenotransplant was more pronounced than in the control [24].

After transplantation of NSC isolated from the brain of 9-12-week human fetuses and cultured *in vitro* into the spinal cord of adult rats with spinal trauma, these cells retain viability for 110 days without immunosuppression, migrate into injured spinal cord of the recipient rats along the nerve fibers and vessels towards the necrotic zones, and differentiate into neurons and glial cells [2].

Human fetal SC implanted into rat spinal cord can differentiate into cholinergic motoneurons; their axons then form contacts with myocytes of medial sural muscles. Axons of transplanted cells are myelinated. Transplantation of NSC leads to normalization of electromyograms of medial sural muscles [14].

Transplants of cultured NSC obtained from a 9.5-week human fetus successfully survived, retain multipotent status, and produce a neuroprotective effect on degenerating neurons in the brain of adult rats subjected to hypoxic hypoxia [1].

Experiments with transplantation of NSC obtained from 9-week human embryos into the lateral ventricle of adult rats showed that these cells possess high plastisity and continue their development in the new environment. Morphogenetic processes underlying this development include migration of nerve and glial cells and differentiation into neurons and glia [3]. A principal possibility of the use of fetal nervous tissue for the treatment of Parkinson disease was first studied on the model of experimental Parkinsonism in lowest primates (marmosets). Transplantation of embryonic dopanimergic neurons into the striatum of experimental animals led to partial recovery of lost functions.

The efficiency of transplantation of NSC isolated from the brain of 13-week human embryos to macaques with experimental deficiency of dopaminergic neurons was evaluated. After 2 months, the locomotion and food intake were restored and tremor disappeared. But after 4 months deterioration of the functional state of animals was observed. Instead of expected accumulation of newly formed neurons immunohistochemical analysis revealed accumulation of donor cells around survived dopaminergic neurons protecting nerve cells from further degradation. It was assumed that transplanted NSC secrete a cocktail of neuroprotective cytokines. If the function of donor cells consists in cytoprotection, the transplantation should be performed at the early stages of the disease for attaining the better therapeutic effect. Authors believe that the decrease in the therapeutic effect is determined by the development of the immune response to the transplant in the absence of immunosuppressor therapy [30].

In some cases, committed precursors, but not SC are more preferable for attaining the therapeutic effect. Transplantation of human NSC is used for correction of functional deficiency in spinal injuries (via restoration of interneuronal contacts, replacement of lost neurons or oligodendrocytes, and production of neurotrophic factors). However, local implantation of human fetal NSC to rats with contusion damage to the spinal cord produced no therapeutic effect, although was not accompanied by its rejection. At the same time, local injection of committed neural progenitor cells from the same source led to their neuronal and glial differentiation with partial functional rehabilitation [33].

Oligodendrocyte precursors isolated from 21-23-week human fetuses and xenografted to the forebrains of newborn shiverer mice with myelinization defect dispersed throughout the white matter and developed into oligodendrocytes and astrocytes. By the 12th week, the host brains showed extensive myelin production and axonal myelination. It was assumed that transplantation of oligodendrocyte

precursors can be use in pediatric practice for the therapy of leukodystrophies and pathological states determined by metabolic disturbances manifesting in myelin deficiency or defects [36].

Diseases of the locomotor system. Analysis of laboratory and clinical results on the use of fetal cells and cells from adult donors for the correction of locomotor disturbances was performed by some authorities.

In adults, possible sources for engineering of the bone tissue are freshly isolated non-fractionated bone marrow, purified and defrosted *in vitro* MSC, differentiated osteoblasts, or modified transfected cells expressing morphogens of the bone tissue. In fetuses, cells of the amniotic fluid, placenta, umbilical cord, and bone tissue can also be used for these purposes. Expansion in culture was maximum in case of least differentiated cells due to their high proliferative activity, while differentiated cells produce most pronounced effect *in vivo*.

An evident advantage of fetal cells isolated from the bone tissue over cells from adult donors was demonstrated by the number of parameters. By the intensity of proliferation, fetal bone cells are comparable with adult MSC, but are far superior to osteoblasts. Expression of cbfa-1, alkaline phosphatase, type I collagen α1 chain, and osteocalcin in fetal bone cells increases after 12-day incubation with osteogenic factors. The efficiency of induction of osteogenic differentiation markers in fetal cells is higher than in MSC and osteoblasts from adult donors. After transplantation to the recipient, fetal cells more actively participate in the formation of the bone tissue than cells from adults and do not induce inflammatory and immunological responses. The process of mineralization of fetal bone cells starts earlier than in MSC and osteoblasts from adults. Primary bone marrow cells are superior to MSC from adult donors by the efficiency of production of mineralized extracellular bone matrix [29].

Allogeneic fetal MSC (32 week gestation) were transplanted *in utero* to the fetus with prenatally diagnosed severe osteogenesis imperfecta. At the age of 9 months, on histological preparations of bone biopsy specimens stained for osteocalcin or osteopontin, 6.8-16.6% XY-positive cells (from male donor) were revealed in the recipient girl. Bone tissue had normal structure with well-formed bone trabeculae. Donor MSC did not induce proliferation of patient T lymphocytes. Complementary bisphosphonate treatment was begun at 4 months. During the first 2 years of life, three fractures were noted. Psychomotor development and growth of the child were normal. Hence, allogeneic fetal MSC can engraft and differentiate into the bone tissue in a human fetus even when the recipient is immunocompetent and HLA-incompatible with the donor [23].

Cardiovascular diseases. Bone marrow, endothelial precursors, MSC, and cardiogenic SC and ESC located in the myocardium can be potential sources of cells for replacement therapy in cardiovascular failure. Cells of the chorionic plate of the fetal part of human placenta express cardiomyocyte-specific genes, *e.g.* cardiac myosin heavy chain 7β, atrial myosin light chain, and cardiac α-actin. These cells also express Csx/Nkx2.5, GATA4, cardiac troponin-I, and connexin-43. During cardiomyogenic differentiation cardiac troponin-I and connexin-43 are located in sites of intercellular contacts. Spontaneous pulsation of cells is observed starting from day of co-culturing with mouse fetal cardiomyocytes, the rate of pulsation attains the maximum on day 12. Contractions of cardiomyocytes are rhythmic and synchronous [28].

Cells with cardiomyogenic potential were also found in the amniotic fluid. Amniotic SC express endothelial (angiopoietin, CD146), smooth-muscle (smoothelin), and cardiomyocytic (Nkx2.5, MLC-2v, GATA4, and β-cardiac myosin heavy chain). Some amniotic SC acquire cardiomyocytic phenotype only during co-culturing with cardiomyocytes from newborn rats [8].

Human fetal aorta also contains vascular progenitor cells, non-differentiated mesenchymal cells expressing endothelial and myogenic markers simultaneously. During *in vitro* culturing in the presence of VEGF-A or PDGF-ββ they give rise to a mixed population of mature endothelial and mural cells. Vascular progenitors embedded into a 3D collagen gel are reorganized into continuous cell cords resembling mature vascular structures. Supernatants of these cells contains angiogenic factors (VEGF-A and angiopoietin-2) and markedly stimulates proliferation of endothelium. After transplantation of a small amount of vascular progenitor cells into ischemic limb muscle of immunodeficient mice, donor cells considerably stimulate neovascularization and prevent the death of endogenous endothelial cells and myocytes, thus ameliorating the clinical outcome from ischemia [20].

A principally new approach to the treatment of congenital heart disease was proposed. It is based on the use of prenatal mesenchymal precursor cells from chorionic villi cultured *in vitro* on a biodegradable matrix structurally identical to the heart valve. The novelty of this approach consists in the use of autologous cells obtained at the stage of embryogenesis for fabrication of the heart valve. Endothelial precursor cells from the umbilical cord blood

were used for endothelialization of the bioprosthesis. The obtained tissue construct by its structural organization, cell phenotype, production of extracellular matrix, and DNA content is identical to native heart valve tissue. Thus, this approach opens prospects of using these bioconstructs for postnatal surgical correction of congenital heart diseases [32].

Liver diseases. Cells therapy can be an alternative approach to the treatment of some liver pathologies. The advantage of the use of SC/progenitor cells consists in the possibility of cell expansion *in vitro* and their genetic modification.

MSC from human fetal liver can *in vitro* differentiate into functional hepatocyte-like cells with an immunophenotype $CD166^{+}CD34^{-}$. On days 21-28 in culture, MSC seeded into a matrigel in the presence of FGF4 and HGF acquire polygonal or round shape. Expression of GATA4, α-fetoprotein, and cytokeratin 18 starts at early terms of induction. Expression of albumin, cytokeratin 18, GTS, and hepatocyte transcription factor HBF1 increases during culturing. Accumulation of glycogen was observed starting from day 14 and peaked on day 28 [17].

Thus, MSC from human fetal liver can differentiate into hepatocyte precursors and then into mature hepatocytes and hepatocyte-like cells carrying hepatocyte markers. Induced hepatocytes and hepatocyte-like cells acquire functional characteristics specific for this cell type.

Ophthalmologic diseases. Retinal precursors of fetal origin can be used for replacement of damaged retinal cells, vision recovery, or prevention of retinal degeneration.

In RCS rats with autosomal recessive pigment retinitis and secondary degeneration of photoreceptors, injection of cells from human umbilical cord into subretinal space preserved structural integrity of photoreceptors and provides visual functions. Transplanted cells did not differentiate into neurons and did not form tumors [25].

For the maintenance of visual function in RCS rats we also used NSC isolated from the brain cortex of 13.5-week human fetus. Unmodified NSC or NSC expressing a transgene GDNF were subretinally injected to animals at the age preceding major photoreceptor loss. On days 90-100 after birth, *i.e.* by the moment when untreated rats exhibit little or no retinal or visual function, recipients retained substantial retinal electrical activity and visual fields with near-normal visual acuity. Transplantation of genetically modified NSC producing GDNF led to even more pronounced functional recovery. Histological examination on day 150 after birth showed that NSC formed a nearly continuous pigmented layer between the neural retina and retinal pigment epithelium, as well as distributed within the inner retina. Host cone photoreceptors were also protected by fetal human NSC. It was hypothesized that transplantation of fetal NSC can be applied in clinical practice for the treatment of some degenerative retinal diseases [13].

Thus, the use of fetal tissues as the source of SC and progenitor cells is promising and preferable for cell therapy. High proliferative and differentiation potential of these cells and the absence of immune reaction to them confirmed by ample experimental data suggest that they can be used for the treatment of various pathologies in humans.

REFERENCES

1. O. V. Podgornyi, I. V. Kheifetz, M. A. Aleksandrova, *et al., Byull. Eksp. Biol. Med.*, **137**, No. 4, 394-398 (2004).
2. G. A. Stepanov, D. O. Karpenko, M. A. Aleksandrova, *et al., Ibid.*, **135**, No. 4, 466-470 (2003).
3. G. T. Sykhikh and V. V. Malaitsev, *Ibid.*, **131**, No. 3, 244-255 (2001).
4. G. Almeida-Porada, D. El Shabrawy, C. Porada, and E. D. Zanjani, *Exp. Hematol.*, **30**, No. 12, 1454-1462 (2002).
5. D. W. Bianchi and N. M. Fisk, *JAMA,* **297**, No. 13, 1489-1491 (2007).
6. E. Cacci, A. Villa, M. Parmar, *et al., Exp. Cell Res.*, **313**, No. 3, 588-601 (2007).
7. J. Chan, K. O'Donoghue, J. de la Fuente, *et al., Stem Cells*, **23**, No. 1, 93-102 (2005).
8. A. Chiavegato, S. Bollini, M. Pozzobon, *et al., J. Mol. Cell. Cardiol.*, **42**, No. 4, 746-759 (2007).
9. Y. Y. Dan, K. J. Riehle, C. Lazaro, *et al., Proc. Natl. Acad. Sci. USA.*, **103**, No. 26, 9912-9917 (2006).
10. A. Darinskas, R. Gasparaviciute, M. Malisauskas, *et al., Mol. Cell. Biol. Lett.*, **12**, No. 3, 422-432 (2007).
11. P. De Coppi, G. Bartsch, M. M. Siddiqui, *et al., Nat. Biotechnol.*, **25**, No. 1, 100-106 (2007).
12. A. Fine, *CMAJ,* **151**, No. 9, 1261-1268 (1994).
13. D. M. Gamm, S. Wang, B. Lu, *et al., PloS ONE*, **2**, No. 3, e388 (2007).
14. J. Gao, R. E. Coggeshall, J. M. Chung, *et al. Neuroreport.*, **18**, No. 6, 565-569 (2007).
15. C. Gotherstrom, A. West, J. Liden, *et al., Haematologica*, **90**, No. 8, 1017-1026 (2005).
16. P. V. Guillot, C. Gotherstrom, J. Chan, *et al., Stem Cells*, **25**, No. 3, 646-654 (2007).
17. N. H. He, W. L. Zhao, Y. M. Wang, *Zhonghua Gan Zang Bing Za Zhi.*, **15**, No. 3, 164-169 (2007).
18. Y. Hu, L. Liao, Q. Wang, *et al., J. Lab. Clin. Med.*, **141**, No. 5, 342-349 (2003).
19. P. S. in't Anker, W. A. Noort, S. A. Scherjon, *et al. Haematologica*, **88**, No. 8, 845-852 (2003).
20. G. Invernici, C. Emanueli, P. Madeddu, *et al., Am. J. Pathol.*, **170**, No. 6, 1879-1892 (2007).
21. M. Kassem, J. S. Burns, J. Garcia Castro, and D. Rubino Munoz, *Cancer Res.*, **65**, No. 20, 9601 (2005).
22. K. Khosrotehrani and D. W. Bianchi, *J. Cell Sci.*, **118**, Pt. 8, 1559-1563 (2005).

23. K. Le Blanc, C. Gotherstrom, O. Ringden, *et al., Transplantation*, **79**, No. 11, 1607-1614 (2005).
24. H. J. Lee, K. S. Kim, E. J. Kim, *et al., Stem Cells*, **25**, No. 5, 1204-1212 (2007).
25. R. D. Lund, S. Wang, B. Lu, *et al., Ibid*., **25**, No. 3, 602-611 (2007).
26. M. Michejda, *Fetal Diagn. Ther.,* **19**, No. 2, 111-112 (2004).
27. M. Mimeault and S. K. Batra, *Stem Cells*, **24**, No. 11, 2319-2341 (2006).
28. K. Okamoto, S. Miyoshi, M. Toyoda, *et al., Exp. Cell Res*., **313**, No. 12, 2550-2562 (2007).
29. D. P. Pioletti, M. O. Montjovent, P. Y. Zambelli, and L. Applegate, *Swiss Med. Wkly.* **136**, Nos. 35-36, 557-560 (2006).
30. D. E. Jr. Redmond, K. B. Bjugstad, Y. D. Teng, *et al., Proc. Natl. Acad. Sci. USA*., **104**, No. 29, 12,175-12,180 (2007).
31. P. Rollini, S. Kaiser, E. Faes-van't Hull, *et al., Blood*, **103**, No. 3, 1166-1170 (2004).
32. D. Schmidt, A. Mol, C. Breymann, *et al., Circulation*, **114**, No. 1, Suppl. 1125-1131 (2006).
33. Y. I. Tarasenko, J. Gao, L. Nie, *et al., J. Neurosci. Res*., **85**, No. 1, 47-57 (2007).
34. T. Terashima, H. Kojima, M. Fujimiya, *et al., Proc. Natl. Acad. Sci. USA*., **102**, No. 35, 12,525-12,530 (2005).
35. D. A. Williams, H. Xu, J. A. Cancelas, *J. Clin. Invest*., **116**, No. 10, 2593-2596 (2006).
36. M. S. Windrem, M. C. Nunes, W. K. Rashbaum, *et al., Nature Medicine,* **10**, No. 1, 93-97 (2004).
37. Y. Zhang, C. D. Li, X. X. Jiang, *et al., Chin. Med. J.,* **117**, No. 6, 882-887 (2004).