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Review

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Stem cells and exosomes: Applications in pediatric surgery

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Abstract

Regenerative medicine is a new branch of science that aims to restore damaged tissue in acquired and congenital tissue or organ injuries. In parallel with advances in tissue engineering, particularly in the last decade, stem cell and stem cell-derived exosome therapies appear promising for some congenital anomalies relevant to pediatric surgery. While results of stem cell therapy are largely based on experimental studies, a limited number of clinical studies have also reported positive results. The purpose of this article is to summarize the properties of stem cells and exosomes and their applications in pediatric surgery and to increase awareness and interest in this topic among young pediatric surgeons.

Keywords: Exosome, pediatric surgery, stem cells.

STEM CELLS AND THEIR TYPES

Stem cells can transform into any type of cell in our body, self-renew, and migrate to damaged tissue or organs. They can form colonies where they migrate. Stem cells also secrete mediators necessary for cell self-renewal in host tissue. Recently, it has been suggested that not the stem cells themselves but the mediators and exosomes they secrete are effective in tissue regeneration. Stem cells are classified as totipotent, pluripotent, multipotent, oligopotent, and unipotent based on their differentiation potential. They are also defined as embryonic, neonatal, or adult stem cells based on their origin (Figure 1). Amniotic fluid during the fetal period and umbilical cord blood or Wharton jelly tissue during the neonatal period are good sources for stem cell extraction.[1-3] Stem cells obtained from these tissues are younger, more proliferative, and less immunogenic. They are easy to obtain and do not pose ethical problems.[4]

Adult stem cells (somatic stem cells) are undifferentiated cells found in differentiated tissues throughout the body. They can be obtained from tissues such as fat, blood, skin, and muscle. Reprogramming an adult somatic cell with transcription factors such as OCT4 (octamer-binding transcription factor 4), SOX2 (SRY-box transcription factor 2), Klf4 (Krüppel-like factor 4), and c-Myc can give it the properties of a pluripotent stem cell. Programmed somatic cells are called induced pluripotent stem cells (iPSCs). The main difference between iPSCs and embryonic stem cells is their more limited differentiation potential. Induced pluripotent stem cells can also be obtained through methods such as nuclear transplantation, fusion, or the use of cell extracts, in addition to reprogramming. Today, iPSCs have many uses, including the development of *in vitro* disease models, drug toxicity studies, the identification of pathophysiological processes, and the understanding of the fundamentals of developmental biology.[1]

Fundamental properties of stem cells

The characteristic of being a stem cell is associated with transcription factors such as

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OCT3-4, SOX2, Klf4, and Nanog, the suitability of the stem cell microenvironment (e.g., fibroblasts and the interleukin-6 family), and the activation of the Wnt signaling pathway. One of the key characteristics of stem cells is their ability to migrate to damaged tissues. Damaged tissue secretes chemokines such as SDF-1 (stromal cell-derived factor-1) and CCL12 (C-C motif chemokine ligand 12). The CXCR4 (C-X-C motif chemochine receptor 4) receptor on the surface of stem cells also binds to SDF-1. Chemokines such as CCL2 (C-C motif ligand 2), interleukin-8, and VEGF (vascular endothelial growth factor), secreted by damaged tissue, also attract stem cells to the tissue. When stem cells administered via vascular route reach the vessels of damaged tissue, they travel outside the vascular system to the damaged area, influenced by molecules such as selectin and integrin. The extracellular matrix in the damaged tissue is broken down by metalloproteinases, allowing the stem cells to move more easily within the tissue.[5]

Another characteristic of stem cells is their ability to differentiate. Stem cells develop into different cells at their migration site, depending on their differentiation potential. Early-stage stem cells, from the zygote to the blastocyst stage, are totipotent. These cells develop both the embryo and the placenta. During the blastocyst stage, the inner cell mass differentiates into cells that will form the embryo (pluripotent stem cells). Adult stem cells programmed to differentiate into a single germ layer, such as bone marrow and mesenchymal stem cells, are called multipotent stem cells. Stem cells that can differentiate into two or more cell lineages in a specific tissue or organ, such as myeloid or lymphoid tissue, are considered oligopotent, while stem cells that differentiate into a single cell type, such as spermatogonial stem cells, are considered unipotent (Figure 1).[1,3,6]

Stem cells have the capacity for self-renewal. This is the ability of a stem cell to divide and ensure that at least one daughter cell remains a fully identical stem cell. This prevents depletion of the stem cell pool. Self-renewal in stem cells can happen in two ways: asymmetrically, where one daughter cell remains a stem cell and the other becomes a progenitor cell, or symmetrically, where both daughter cells either remain stem cells or both become progenitor cells. Symmetrically,

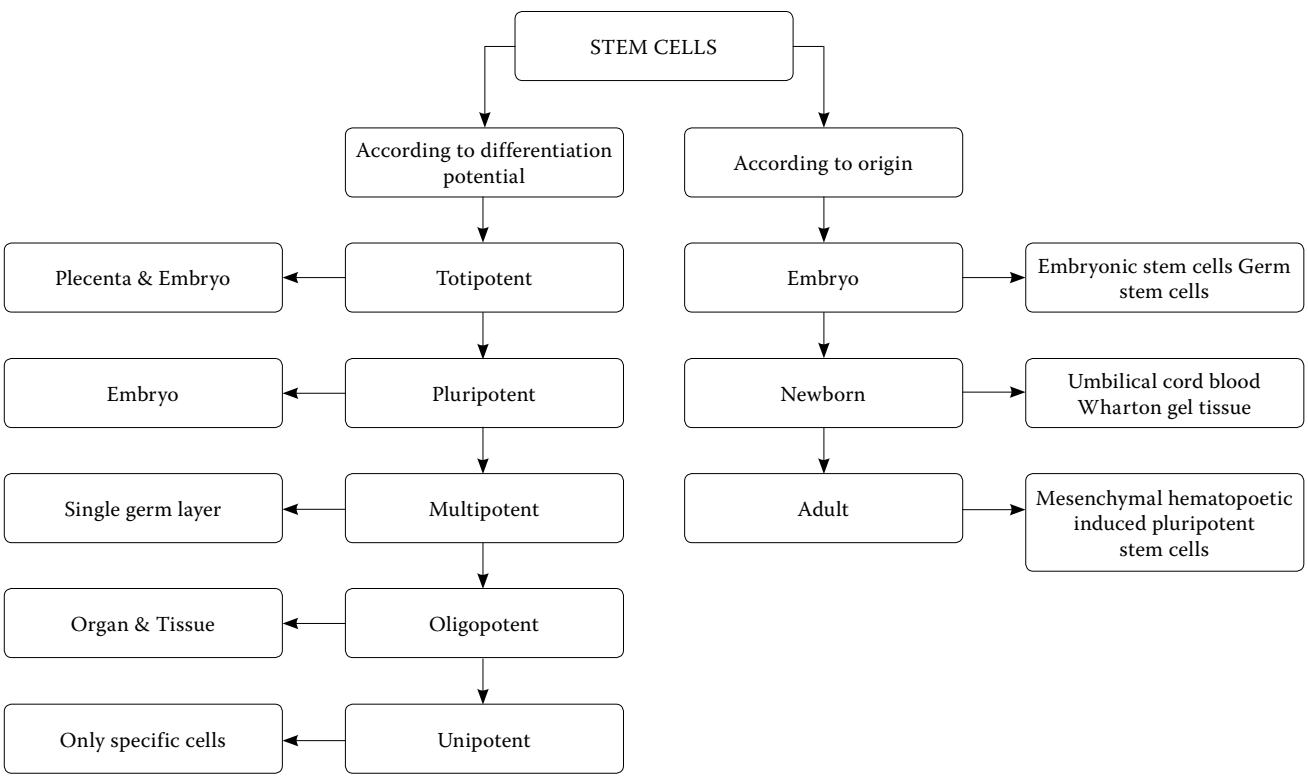

Figure 1. Classification of stem cells according to their differentiation potential and origin.

this division is used to preserve the stem cell pool during embryonic development or during severe tissue injury. Asymmetric or symmetric division is controlled by intracellular transcription factors or the stem cell microenvironment.[1,7]

It is important for stem cells to be able to form colonies where they migrate. Colony formation is defined as the generation of multiple stem cells from a single stem cell. The ability to form colonies is essential, particularly when propagated in culture, to maintain their viability and allow for freezing and thawing as needed. If multiple cell types are present in the culture, colony formation may be negatively impacted.[1,3,6]

Exosomes and their properties

Recently, exosome therapies have become a focus of interest in tissue regeneration. Contrary to initial beliefs about stem cell therapies, mesenchymal stem cells do not directly generate new tissue, but the packaged exosomes they secrete into the environment have a direct impact on tissue regeneration.[8]

Exosomes are a subgroup of extracellular vesicles, ranging in size from 30 to 150 nm in diameter. They are secreted outside the cell as packages surrounded by a lipid bilayer membrane. They contain molecules such as proteins, lipids, nucleic acids (DNA, mRNA, and miRNA), growth factors, and cytokines. Their primary function is to facilitate communication between cells at close and distant locations. They can be released from many living cells, such as stem cells or tumor cells. These nanovesicles, which present antigens or share genetic information through mRNA and miRNA, are involved in many physiological and pathological processes, such as tissue repair and tumor metabolism. They are more stable than cell-based therapies. They can maintain their stability for nine months at –152 °C. Because they are not cell-based, they are less likely to cause immune rejection. They are nontoxic and have no aneuploidy. Their small size makes it easier to reach damaged tissues.[9-11]

Stem cell and exosome administration routes

Stem cells and exosomes can be administered intravenously, intraperitoneally, directly into tissue, or into amniotic fluid. However, in many clinical and experimental studies, stem cells are administered intravenously. An important question is how many of the administered stem cells or exosomes reach the damaged tissue if administered intravenously. Furthermore, the long-term effects of stem cells administered into the systemic circulation on other healthy tissues and organs are not fully known. Direct administration into damaged tissue may make it more difficult for stem cells to attach and survive. Animal studies constitute the majority of intraperitoneal administrations in the literature. With intraperitoneal administration, stem cells or exosomes also enter the systemic circulation.

Concerns about stem cell therapy?

Due to their pluripotency, embryonic stem cells carry a risk of developing tumors (teratoma) in host tissue. The activity of p53 (tumor suppressor gene) is important for genomic stability. Its activity is lower in pluripotent stem cells than in normal somatic cells. Therefore, pluripotent stem cells have the capacity to divide and self-renew more rapidly. However, the absence or mutation of p53 increases the risk of cancer. Spontaneous mutations, particularly when iPSCs are grown *in vitro* or when mutant cell colonies are selected for treatment, increase this risk. Therefore, the presence of mutations in p53 should be investigated before stem cell therapy.[1,12]

A systematic meta-analysis of 36 studies applied mesenchymal stem cell therapy in various clinical conditions such as myocardial infarction, heart failure, ischemic stroke, and Crohn's disease and found no increased susceptibility to tumor development or infections associated with mesenchymal stem cell therapy. Transient fever has only been reported as significant in patients during intravenous administration. However, in this meta-analysis, patient follow-up periods ranged from two weeks to five years. Therefore, it is not possible to make a definitive conclusion.[13] Another question is whether the transplanted cells have an immunogenic effect. Immunogenic effects are not expected with autologous cell transfer. While immune reactions may occur with allogeneic mesenchymal stem cell transfers, the risk is reported to be very low.

Stem cell and exosome applications in pediatric surgery: Where do we stand?

While studies on stem cell therapy have focused primarily on necrotizing enterocolitis

(NEC), Hirschsprung disease, and congenital diaphragmatic hernia, there are also studies on esophageal atresia, gastroschisis, testicular torsion, and liver cirrhosis associated with biliary atresia. Paulo de Coppi's studies on stem cell therapy for congenital anomalies such as congenital diaphragmatic hernia and Hirschsprung disease hold a significant place in the literature.[4,14-16] While the majority of these studies are still in the experimental phase, they offer promising results.

Necrotizing enterocolitis, which is particularly common in premature infants, continues to be a pathology with high morbidity and mortality. The challenges in early diagnosis and treatment have led to the search for innovative treatments. Experimental NEC models have shown a decrease in the number of stem cells that provide intestinal epithelial regeneration.[17] Numerous studies have shown that bone marrow-derived mesenchymal stem cells (BM-MSCs), amniotic fluid-derived mesenchymal stem cells (AF-MSCs), or exosomes in experimental NEC models maintain intestinal barrier functions, repair damaged intestinal epithelium, suppress inflammation, and reduce apoptosis.[18,19] These effects in the intestine are thought to be related to the activation of the Wnt/β-catenin pathway, which increases Lgr5+ expression. As a result of stem cell differentiation into goblet cells, mucin secretion increases and the intestinal defense mechanism is strengthened.[19] Additionally, increased cyclooxygenase 2 activity in the intestine increases prostaglandin synthesis, resulting in an anti-inflammatory effect.[18-20] In summary, stem cells derived from different sources reduce the frequency and severity of NEC, accelerate epithelial regeneration, and suppress inflammation.[21,22] Studies have shown that BM-MSCs and AF-MSCs secrete different proteins; AF-MSCs are effective in cell development and growth, while BM-MSCs are effective in regulating immunity. Therefore, AF-MSC treatment is effective in the prophylaxis of NEC, while BM-MSC treatment does not have the same effect.[23,24] Exosomes have also been shown to be similarly effective to stem cell applications in an experimental NEC model, exerting their effects via the Wnt/β-catenin pathway.[20,25]

Another area where stem cell research in pediatric surgery is focused is Hirschsprung disease. The goal is to restore bowel motility by transplanting enteric neural stem cells (ENSCs) into the aganglionic segment of the intestine. However, because autogenous transplants require intestinal resection for cell collection, allogeneic or iPSCs may be more appropriate. While studies have demonstrated migration and integration of transplanted stem cells into the myenteric and submucosal plexuses, functional intestinal contractility depends on the transplanted cells establishing synaptic connections. In a very recent study, ENSCs were transplanted into mice with Hirschsprung syndrome. The transplanted cells formed neoganglia in the aganglionic colon, migrated through the muscle layers, and produced contractions in the intestinal smooth muscle. Thus, it was demonstrated that the transplanted cells also developed synaptic connections.[26] In another study, human ENSC progenitors were transplanted into the aganglionic colon, and intestinal contractility was observed. This result supports the existence of synaptic connections demonstrated in a previous study.[16]

In congenital diaphragmatic hernias, the concept of closing the defect with a myogenic patch that exhibits muscle function and regenerating the hypoplastic lung could significantly reduce the morbidity and mortality of the anomaly. The presence of multipotent stem cells in the distal airway niches of the lung is known. When stem cells are injected into damaged lungs of mice, they have been shown to develop bronchioles, alveoli, and pulmonary vessels, increasing lung surface area and branching morphogenesis.[27-29] Studies support the development of alveolar and vascular structures through the various growth factors and extracellular vesicles (exosomes) secreted by stem cells.[30] Amniotic fluid-derived mesenchymal stem cells have also been shown to increase surfactant expression in hypoplastic lungs.[31] They can often be applied as transamniotic stem cell therapy in congenital diaphragmatic hernia. Mesenchymal stem cells are easy to obtain from amniotic fluid and are promising for the treatment of pulmonary hypoplasia and hypertension.[29] Transamniotic stem cell therapy is also considered promising for neural tube and abdominal wall defects. In an experimental gastroschisis model, AF-MSCs were shown to reduce intestinal inflammation associated with gastroschisis.[32] Furthermore, stem cells and exosomes found in amniotic fluid specific to certain

congenital anomalies can be used as markers for early diagnosis of disease.[33,34]

One of the ultimate goals in regenerative medicine is the development of tissues or organs from three-dimensional organic or inorganic scaffolds by stem cells. These organoids may be a potential future solution for long-segment esophageal atresia, esophageal loss due to corrosive agents, or short bowel syndrome. In one study, mesenchymal stem cells were obtained from the adipose tissue of patients with long- and short-segment esophageal atresia, then expanded *in vitro* and implanted onto polyurethane tubular scaffolds. It has been demonstrated that the stem cells maintain their viability on the tubular scaffolds, can express genes, and secrete cytokines. This observation may enable the future generation of a new esophageal segment in patients with long-segment esophageal atresia using stem cells derived from their own adipose tissue.[35]

Another area where experimental studies have focused is related to testicular damage and infertility. In 1994, spermatogonial stem cells were first isolated from donor mice and injected into the seminiferous tubules of sterile mice, and the spermatogonial stem cells formed colonies in the seminiferous tubules. Furthermore, sterile mice were shown to be able to produce mature spermatoids.[36] In 2003, it was demonstrated that female mouse embryonic stem cell cultures could undergo meiosis and form follicle-like structures.[37] In 2007, BM-MSCs were also shown to be able to differentiate into male germ cells.[38]

Recently, the importance of exosomes released from testicular cells (prostatosomes and epididymosomes) in maintaining normal spermatogenesis and steroidogenesis has been emphasized. Sertoli cell-derived exosomes, in particular, are thought to promote the differentiation of spermatogonial stem cells, while immature Sertoli cell-derived exosomes are thought to be important for the survival of Leydig cells. The ability of exosomes to cross the testicular-blood barrier is a therapeutic advantage.[11] Şimşek and Şencan[39] administered exosomes derived from adipose-derived mesenchymal stem cells intratesticularly in a testicular torsion model and histologically demonstrated that ischemia-reperfusion injury was reduced and the apoptotic pathway was suppressed. The authors then investigated the effects of exosomes on cell death pathways and spermatogonial stem cells in the testicular torsion model. The authors observed that the necroptotic pathway was suppressed along with the apoptotic pathway, and the number of spermatogonial stem cells increased in the treatment group.[40]

Experimental studies on stem cell therapy in pediatric surgery continue in many pediatric urological conditions and in burn patients. However, clinical studies are extremely limited and mostly consist of case reports.[41,42] A limited number of clinical studies have reported the benefit of stem cell therapy in the management of NEC and biliary atresia-associated cirrhosis.[43]

In conclusion, the vast majority of stem cell and exosome applications in disease groups relevant to pediatric surgery are still in the experimental phase. However, stem cell and exosome therapy have the potential to change the surgical paradigm in the future for congenital anomalies and organ damage. Some promising results have already been obtained in NEC and Hirschsprung disease. However, standardizations such as the dose and frequency of stem cell or exosome administration, route of administration, and monitoring criteria need to be developed. Clinical trials and application indications should be conducted within ethical frameworks and in accordance with the Guideline for Clinical Research and Clinical Trials Using Tissues and Cells of the Ministry of Health of the Republic of Türkiye.

Future perspectives

Stem cell and exosome therapies may become standard in the future for many diseases currently in the experimental phase. The combination of genetic editing (CRISPR, clustered regularly interspaced short palindromic repeats) and stem cell therapies may offer a solution for some genetic diseases. Microfluidic chip technology (organ-on-a-chip) can better understand the pathophysiology of diseases and develop personalized treatments. Three-dimensional bioprinting and organoids can be used to produce tissues or organs in the laboratory. Exosomes can be used as targeted drug carriers. Because exosomes are very small and can cross the blood-brain barrier, they may offer advantages in the treatment of neurological diseases. Furthermore, the different molecules they contain may allow them to be used as biomarkers in the early diagnosis of diseases.

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