STEM CELLS AND THEIR OUTSTANDING CONCERNS

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ABSTRACT

Stem cells have captured considerable scientific and clinic interest because of their potential to renew themselves and to differentiate into one or more adult cell types. Thus stem cells have been recognized as a potential tool for the development of innovative therapeutic strategies in different disease disorders. Stem cells can be discriminating based on their differentiated potential as totipotent, pluripotent, multipotent or unipotent cells. There are in general three types of stem cells: embryonic, fetal and adult stem cells. While embryonic stem cell therapy has a lot of ethical concerns due to their obtaining but also unlimited proliferation and uncontrolled differentiation, fetal and adult stem cells have been used in the treatment of different diseases. The bone marrow, peripheral blood and umbilical cord blood are ideal sources of adult stem cells because there are easily accessible and contain two types of stem cells: hematopoietic stem cells giving rise to all blood cell types and mesenchymal stem cells differentiating into cells of mesodermal lineage. This review describes the general characteristics of these stem cell populations and their current applications in regenerative medicine. Additionally induced pluripotent stem cells generated through the reprogramming of differentiated adult cells are described.

KOMÓRKI MACIERZYSTE I ICH MOŻLIWOŚCI ZASTOSOWANIA TERAPEUTYCZNEGO

STRESZCZENIE

Możliwość wspomagania regeneracji nieodwracalnie uszkodzonych tkanek i narządów za pomocą transplantacji komórek macierzystych skupia uwagę środowisk medycznych i opinii publicznej. Komórki macierzyste mają zdolność do samo-odnowy oraz do różnicowania się w dojrzałe komórki struktur całego organizmu. Na określonych etapach rozwoju osobniczego wykazują różny stopień ograniczenia potencjału do dalszego różnicowania, od komórek totipotencjalnych (zygota), poprzez pluripotencjalne (komórki zarodkowe) do multipotencjalnych lub unipotencjalnych (komórki somatyczne). Zarodkowe komórki macierzyste, z uwagi na nieograniczone zdolności namnażania i pluripotencjalność wydają się najbardziej optymalne do zastosowania w terapiach regeneracyjnych, lecz zastrzeżenia natury etyczno-moralnej pozyskiwania tych komórek, a także możliwość ich niekontrolowanej proliferacji po przeszczepieniu w organizmie biorcy skłoniły badaczy do poszukiwania alternatywnych źródeł komórek macierzystych, jakimi są tkanki dojrzałego organizmu. Badania ostatnich lat wykazały, iż w dojrzałych tkankach somatycznych znajdują się pluripotencjalne komórki macierzyste, co więcej odkrycia Takahashi i Yamanaka w roku 2006 r udokumentowały możliwość przekształcenia zróżnicowanych komórek izolowanych od osobników dorosłych w komórki pluripotencjalne. Zainicjowanie nowatorskich metod badawczych, których celem będzie opracowanie skutecznych sposobów przeszczepiania somatycznych komórek macierzystych w celach regeneracyjnych jest nadzieją w terapii wielu nieuleczalnych schorzeń.

Stem cells have been fascinating for scientists and clinicians due to their undifferentiated state that can give rise to a highly specialized cell type. Only recently stem cells have been recognized as a potential tool for the development of innovative therapeutic strategies.

Stem cells are defined by two properties: the capacity to renew themselves and ability to differentiate into most mature cell types. The stem cells retains its state either through asymmetric cell division, giving rise to one stem cell and one progenitor cell that may differentiate into diverse range of specialized cell types, or by reversal of a progenitor cell back to a stem cell after symmetric division (Morrison i Kimble).

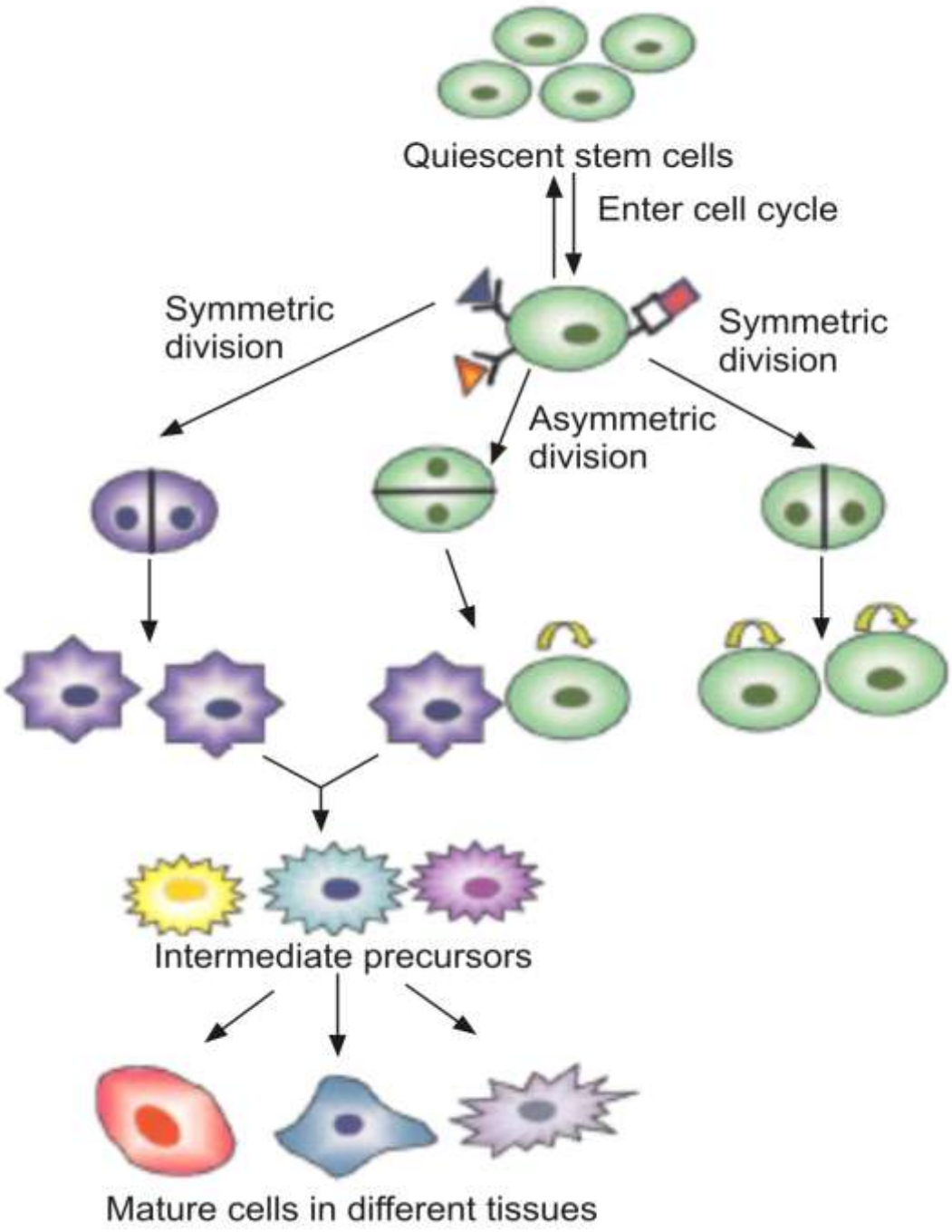

Fig.1. Schematic representation of symmetric and asymmetric divisions of stem cells

Stem cell commitment

Based on their differentiating potential, stem cells appear to be at four levels of commitment:

- **totipotent stem cells** with their capacity to differentiate into cells of the three germ layers (ectoderm, mesoderm and endoderm), giving rise to a functional organism. The cells deriving from an early progeny of the zygote up to the eight cell stage of the morula are defined as totipotent (Oligny).
- **pluripotent stem cells** that can differentiate into cells of all tissue lineages but not into annexes as such zygote. Embryonic cells in the inner cell mass of the blastocyst are truly pluripotent cells (Sjögren, Hardarson i Andersson)
- **multipotent stem cells** capable of yielding a more restricted subset of cell lineages within the tissue of its origin. Stem cells located in the fetal and adult tissues have multipotent abilities.
- **unipotent stem cells** that can produce cell types identical to itself. The example of unipotent cell is the muscle stem cell

Ontogenic development of stem cell

Another type of stem cell classification is based on the developmental stage from which they are isolated i.e. embryonic stem cells, fetal stem cells and adult stem cells.

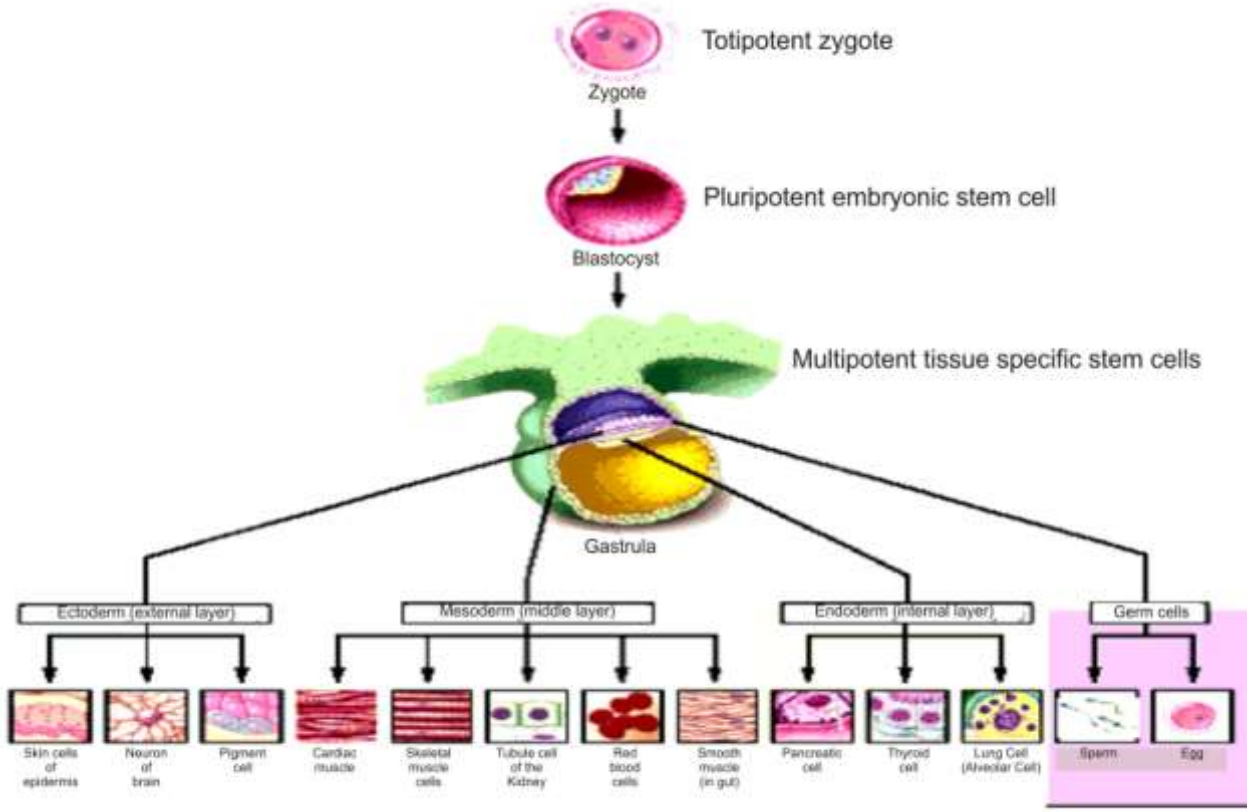

Fig.2. Scheme of stem cell development and their differentiation into different tissues.

Embryonic stem cells (ESC) have unlimited differentiation potential. They may differentiate into virtually any cell type in the adult body. Embryonic stem cells can be kept proliferating extensively *in vitro* using different culture system i.e. feeder layer of primary murine fibroblasts that sustain continuously undifferentiated ESC or mixture of cytokines or growth factors to stimulate cell replication. Embryonic stem cells are an attractive source for tissue regeneration however developmental "immatureness" connected to their genetic and epigenetic instability and neoplastic transformation as well as difficulties in stable directional differentiation can make their practical application quite difficult (Thomson, Itskovitz-Eldor i Shapiro) (Fujikawa, Oh S. H i Hatch).

Fetal stem cells (FSC) have more limited differentiation potential than embryonic stem cells due to their developmental commitment. However, crossing lineage barriers was already widely documented. This is observed generally *in vitro* and emerging role of the microenvironment in determining and maintaining their developmental potential is suggested. Fetal stem cells derive from fetuses or perinatal (placental) tissues. These cells are higher in number, expansion potential and differentiation abilities if compared with stem cells from adult tissues. In contrast to adult stem cells, FSC are less mature due to their specific postnatal ontogenic position and therefore les immunogenic. The ethical concerns surrounding stem cell isolation from fetuses draw great interest of researchers to placental tissues. Among placental tissues multipotent stem cells have been isolated from umbilical cord blood, amniotic and chorionic membranes, amniotic fluid and umbilical cord matrix (Wharton jelly). Moreover, besides multipotent stem cells embryonic –like stem cells have been found in the umbilical cord tissues (McGuckin, Forraz i Allouard) (Habich, Jurga i Markiewicz) (Jo, Kim i Park). A major advantage of placenta-derived cells is their easy accessibility, the non-invasive nature of collection and lack of ethical barriers to their procurement.

Adult stem cells (ASC) are rare cells thought to be present in all tissues and responsible for maintaining the homeostasis of the specific tissue. Adult stem cells are partially committed stem cells localized in specific stromal niches. They are vital for continuously renewing tissues and play an important role for recovery from injury. Adult stem cells originate during ontogenesis and remain in a quiescent state as the local stimuli induce their cycle recruitment and migration. Thus, niche microenvironment with physical contact and chemical relation among stem cells, stromal cells and extracellular matrix components induce ASC differentiation and their self-renewal.

At the beginning of this century it was assumed that the differentiation potential of ASC is already determined and strictly tissue specific. Recent studies have confirmed that ASC have high plasticity i.e. the complex ability to cross lineage barriers and adopt the expression profile and functional phenotypes of the cells that are typical of other tissues. The plasticity can be explained by trans-differentiation. The direct trans-differentiation is the acquisition of the identity of a different phenotype through the expression of the gene pattern of other tissue. Indirect trans-differentiation refers to the achievement of a more primitive state and the successive differentiation to another cell type. There is an evidence that trans-differentiation is involved in injury repair in other districts, damaged cells or sustaining cellular turn over. The migration, differentiation and proliferation are mediated by the tissue, degree of injury and stem cells involved. Damaged tissue releases factors that induce stem cells homing. The microenvironment determines gene activation and a functional reaction on stem cells.

Adult stem cells are known to be present in adult tissues as multipotent or unipotent stem cells. However, in the recent year's pluripotent ASC were found in the spinal cord, fat tissue, peripheral blood and connective tissue of various organs (Leeb, Jurga i McGuckin). The main drawback of ASC is their low abundance in adult tissues and organs, and also the limited life-span after their isolation and culture *in vitro*. Thus the enormous potential of

unlimited growth and differentiation potential ability of embryonic stem cells has still gain a lot of interest of scientists all other the world and the majority of stem cell research was focused on ESC and their pluripotency.

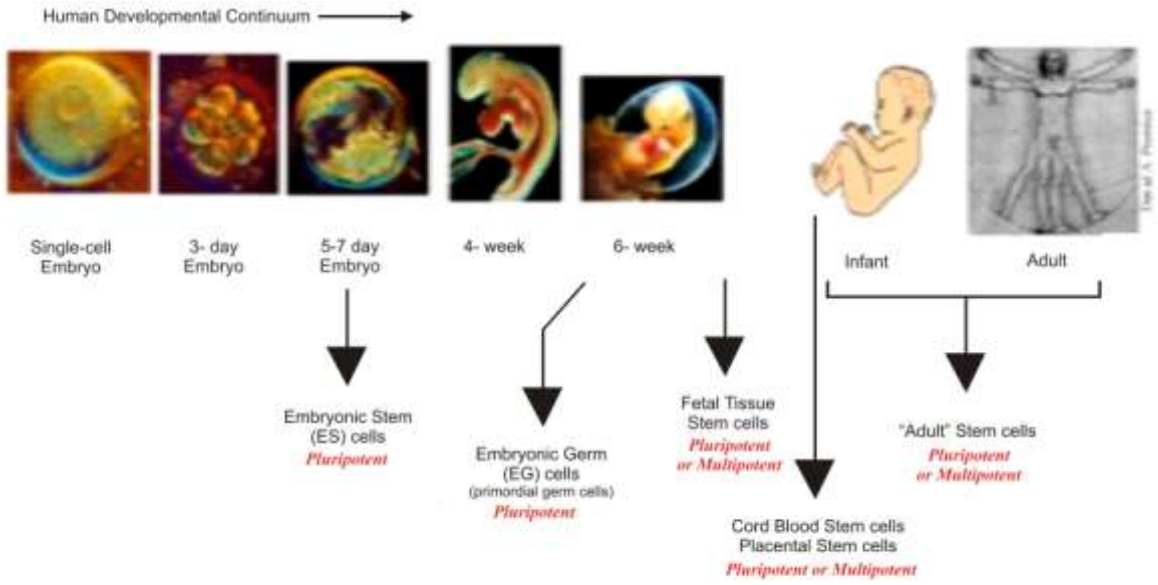

Fig.3. Stem cells in the development of living organism

Induced pluripotent stem cells

Recently, a new source of stem cells – induced pluripotent stem cells (iPS cells) has been reported. In 2006 Takahashi and Yamanaka established a method by which they reprogrammed the adult cells into pluripotent state (Takahashi i Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors.). Successful reprogramming of adult cells was made through retroviral transduction with four transcription factors: Oct3/4, Sox2, c-myc and akf4 into mouse fibroblasts, followed by generation of iPS cells from human fibroblasts using human orthologs of the same set of transcription factor-encoding genes (Takahashi, Tanabe i Ohnuki). These transcriptional factors are highly expressed in embryonic stem cells so the generated iPS cells we similar to ESC in morphology, epigenetic status, proliferation, differentiation and surface marker expression..

Since Takahashi and Yamanaka discovery, the technique for iPS cell generation has been optimized and reproduced in a number of different ways. Induced pluripotent stem cells have been generated using a number of different gene transfer methods, including viral vectors and non-viral plasmids and recently by direct recombinant proteins (Kiskinis i Eggan).

The landmark discovery that lineage-restricted cells can be reprogrammed directly to a state of pluripotency has opened a new era in the field of regenerative medicine. The rapid progression of the iPS cell technology demonstrates the intense interest as a potential application in disease therapy. In contrast to ESC, human iPS cells are not burdened with

ethical, social and religious concerns regarding the use of human embryos. They can be derived from the same individual to be treated thus avoiding immune rejection after their transplantation. The potential of iPS cells is enormous but many obstacles remain before their medical applications can be fully realized. Genomic and epigenetic analysis of these cells generated using current techniques reveal abnormalities that may affect their safe use

However, recent developments allow using iPS cells to construct disease models in vitro for drug screening and for elucidating mechanisms of disease pathogenesis.

Adult stem cells used in clinical therapy

While ES or iPS cell therapy has been riddled with many problems i.e. teratoma formation (germ cell tumors comprising several cell types) adult stem cells have long been used in the treatment of various diseases. The easily accessible sources of adult stem cells are bone marrow, peripheral blood and umbilical cord blood. They harbor two types of somatic stem cells: hematopoietic stem cells and mesenchymal stem cells.

Hematopoietic stem cells (HSC), first identified by Mc Cullough and Till (Mcculloch i Till) are capable of differentiating into all myeloid and lymphoid blood cell progeny. This property of HSC prompted to clinical use them in patients with hematopoietic diseases such as leukemia and myeloid hypoplasia or immunocompromized patients after radiation and/or chemotherapy to treat bone marrow failure. However in the last two decades it was shown that HSC not only commit to their natural lineages but they are able to differentiate into parenchymal cells of most tissues (Petersen, Bowen i Patrene) (Harris, Herzog i Bruscia) (Yoon, Wecker i Heyd). The concept of HSC plasticity opened a new era for HSC to be useful therapeutic tool for otherwise incurable diseases.

Mesenchymal stem cells (MSC) are another type of somatic stem cells. They were discovered in bone marrow by Friedenstein as rapidly growing cells capable of differentiating into various mesodermal lineages including osteoblasts, chondrocytes and adipocytes (Friedenstein, Chailakhjan i Lalykina). Except bone marrow, MSC were also found in all postnatal tissues (Bianco, Robey i Simmons). The majority of MSC are bipotent or unipotent cells however it remains under debate whether MSC can differentiate into cells of non-mesenchymal origin. Moreover they have captured considerably scientific interest because of their potential to limit tissue injury. A number of studies have shown that MSC display unique immunomodulating capacities, anti-inflammation properties and trophic effects (Bartholomew, Sturgeon i Siatskas) (Le Blanc i Ringdén) (Oh, Kim i Shin) (Ankrum i Karp).

Several clinical reports on MSC-based disease treatment have been published in the past decade. In recent years therapeutic applications of MSC have been conducted in a variety of diseases including myocardial infarcts. Different types of neurological disorders autoimmune diseases, juvenile diabetes or GvH disease. The therapeutic effect of MSC is mostly related to their potency of modulating local environment by secreting various factors i.e. cytokines, growth factors, anti-fibrotic or anti-inflammatory mediators (Ghannam, Bouffi i Djouad).

MSC are reported to elicit neuroprotective effects via the release of neurotrophic factors (Crigler, Robey i Asawachaicharn). Transplantation of MSC can exert this effect in retinopathies. The inherent secretion of neurotrophic factors by engrafted cells has generated interest in the glaucoma as impaired neurotrophin support is strongly implicated in retinal ganglion cell death. Stem cell grafting in experimental models has been also effective for the replacement of photoreceptors (Bull i Martin). Finally, the discovery of a population of

proliferating cells in the mammalian retina has raised the possibility of harnessing endogenous retinal stem cells to elicit retinal repair. It seems that stem cell transplantation holds the promise of fulfilling major needs in tissue repair of various disorders including retinal neurodegenerating diseases. However more research needs to be done until this intervention will turn in a routine therapy for patients.

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