MATIČNE ĆELIJE – OPŠTI PREGLED: OD RAZVOJNIH HEMOBIOLOŠKIH KONCEPATA DO *(AUTO)GRAFTING*-a U KLINIČKOJ PRAKSI

PO IZBORU UREDNIKA

EDITOR'S CHOICE

A STEM CELL OVERVIEW – FROM EVOLVING HEMOBIOLOGICAL CONCEPTS TO (AUTO)GRAFTING IN CLINICAL PRACTICE

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SAŽETAK

Konvencionalna transplantacija matičnih ćelija hematopoeze (MĆH) je dobro poznat metod lečenja brojnih stečenih i urođenih poremećaja hematopoeze, poremećaja imunskog sistema, kao i određenih metaboličkih oboljenja. Matične ćelije (MĆ) se mogu definisati kao ćelije koje imaju sposobnost samoobnavljanja i koje poseduju visoki proliferativni kapacitet, kao i potencijal da se diferentuju u funkcionalno kompetentne zrele ćelije. MĆ se mogu podeliti na embrionalne MĆ (EMĆ) i tkivno operdeljene, odnosno adultne MĆ – kao što su one iz kostne srži, periferne krvi i iz krvi pupčane vrpce, kao i druge ne-hematopoetske odnosno somatske ćelije. Kod odraslih, MĆ se obično smatraju ograničenim po pitanju njihovog regenerativnog potencijala i potencijala diferentovanja, dok su EMĆ „prave" totipotentne/pluripotentne ćelije, jer imaju sposobnost da se razviju i u endoderm, ektoderm i mezoderm – tj. u sva tri tipa embrionalnog tkiva u ljudskom organizmu. Među MĆ koje se potencijalno mogu presaditi, ove ćelije predstavljaju vrstu koja najviše obećava, ali i koja je i najkontroverznija. Manje zrele ili primitivnije MĆH imaju potencijal da se diferentuju, ne samo u sve vrste ćelija krvi, već i u neke vrste somatskih ćelija (plastičnost MĆ). U različitim kliničkim uslovima, transplantacija nezrelih (primitivnih) MĆ dovodi do repopulacije kostne srži primaoca transplantata, uz kasniju potpunu, stabilnu i dugoročnu rekonstituciju hematopoeze. Imajući u vidu da su primitivne ćelije takođe sposobne za ukalemljenje (*homing*) u različita tkiva, autologna implantacija MĆ u oštećeno i/ili ishemično područje indukuje njihovo naseljavanje i sledstveno transdiferentovanje u ćelijske linije organa domaćina, uključujući i neovaskularizaciju. Stoga su one klinički primenljive u oblasti regenerativne medicine, u lečenju tkivnih oštećenja miokarda, mozga, krvnih sudova, jetre, pankreasa i drugih tkiva. Svrha ovog pregleda jeste rekapitulacija ključnih otkrića u oblasti istraživanja MĆ, koja je u fazi ubrzanog razvoja. U radu je dat i pregled primene MĆ u tzv. konvencionalnim transplantacijama i u regenerativnoj medicini. Uz to, dat je i sažet kritički osvrt na naša sopstvena istraživanja u oblasti MĆ.

Ključne reči: matične ćelije, plastičnost matičnih ćelija, transplantacija, regenerativna medicina

ABSTRACT

Conventional hematopoietic stem cell transplantation is a well-known treatment method for numerous acquired and congenital hematopoietic disorders, disorders of the immune system, as well as certain metabolic disorders. Stem cells (SCs) can be defined as cells capable of self-renewal with a high proliferative capacity and the potential to differentiate into functionally competent mature cells. Stem cells can be divided into embryonic SCs (ESCs) and tissue-specific or adult SCs – such as bone marrow (BM) stem cells, peripheral blood (PB) stem cells, and SCs derived from umbilical cord blood (UCB), as well as other non-hematopoietic or somatic SCs. SCs in adults are characteristically considered to be restricted in their regenerative and differentiative potential, while embryonic stem cells are 'true' totipotent/pluripotent cells, due to their ability to develop into endoderm, ectoderm, or mesoderm – all three embryonic tissue types in the human body. They are the most promising, but also the most controversial type of potentially transplantable SCs. Immature hematopoietic SCs have the potential of differentiating, not only into all blood cells, but also into some somatic cell types (SC plasticity). In different clinical settings, the transplantation of immature stem cells leads to the repopulation of recipient bone marrow, with subsequent complete, stable, and long-term reconstitution of hematopoiesis. Given that immature stem cells are also capable of homing to different tissues, autologous stem cell implantation into a damaged and/or ischemic area induces their colonizing and consecutive transdifferentiating into cell lineages of the host organ, including neovascularization. Thus, they are clinically applicable in the field of regenerative medicine for the treatment of myocardial, brain, vascular, liver, pancreatic, and other tissue damage. The purpose of this overview is to recapitulate the key developments in the rapidly evolving area of stem cell research, as well as to review the use of SCs in conventional transplantations and in regenerative medicine. Additionally, a brief critical evaluation of our own stem cell research will be summarized.

Key words: stem cells, SC plasticity, transplantation, regenerative medicine

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UVOD

Multiciklična i trajna citopoeza jeste proces razvoja i ekspanzije velikog broja progenitora i prekursora, kao i zrelih ćelija, iz malog broja nediferentovanih (primitivnih) matičnih ćelija (MĆ), *in vivo* ili *ex vivo* [1–3]. Uopšteno gledano, MĆ obezbeđuju stabilnu homeostazu u svakom sistemu koji produkuje tkiva. Dugo se verovalo da su samo embrionalne MĆ (EMĆ) totipotentne/pluripotentne, pošto je plastičnost MĆ od ključnog značaja za rani razvoj. Stoga se dugo vremena smatralo da ćelijsku totipotentnost/pluripotentnost poseduju samo specifične EMĆ. No, najprimitivniji odeljci MĆ kod odraslih takođe imaju gotovo „neograničeni" potencijal samoobnavljanja, kao i sposobnost da prelaze u druge ćelijske linije (inter-sistemska plastičnost) [2–6].

Tokom citopoeze, odigravaju se uravnoteženi procesi složenog sistema interaktivnih supstanci, citokina, faktora rasta i inhibitora. Delimični ili potpuni gubitak uravnotežene regulacije može dovesti do nekontrolisanog rasta ćelija ili njene smrti. Vremenom, odnosno starenjem, aktivnost MĆ opada, rezultujući manjim regenerativnim potencijalom ćelija i tkiva, te redukcije i sposobnosti obnavljanja organa, što se manifestuje u vidu bolesti odnosno defekata u tkivima, uključujući tu i karcinome [2–4].

Hematopoeza je dinamički hemobiološki proces u kojem se velika količina svih vrsta krvnih ćelija nastaje iz populacije primitivnih MĆ hematopoeze (MĆH). One se distribuiraju u različite hematopoetske odeljke u organizmu tokom fetalnog razvoja i adultnog života. Kostna srž odraslih, kao primarna lokacija, ima visoki potencijal diferentovanja u pluripotentne i opredeljene progenitore, koji se na kraju transformišu u različite zrele ćelije krvi, neophodnih za obnavljanje krvi i za borbu protiv infekcija [7–15].

Unutrašnji genetski putevi kontrolišu hematopoezu. Osnovni genetički putevi regulišu interaktivne spoljašnje signale iz ekstracelularnog matriksa i ostale signalne molekularne puteve, kao i mikrosredinu stromalnih ćelija [2,11–14]. Uloga stromalnih ćelija, uključujući makrofage, fibroblaste, dendritske, endotelske i druge ćelije, jeste da stimulišu MĆ. Naime, ove stromalne ćelije stimulišu MĆ oslobađanjem specifičnih faktora rasta, kao što su *Flt3-ligand, c-kit-ligand* ili *SC-factor*, kao i interleukini, faktor stimulacije kolonije granulocita-makrofaga (engl. *granulocyte-macrophage colony-stimulating factor – GM-CSF*) i faktor stimulacije kolonija granulocita (engl. *granulocyte colony-stimulating factor – G-CSF*). Uz lučenje citokina, stromalne ćelije regulišu i adheziju molekula MĆ i omogućavaju im da ostanu u kostnoj srži ili da migriraju u ono područje gde je dati tip ćelije potreban [14–19].

INTRODUCTION

Multicyclic and permanent cytopoiesis is the process of cell development or expansion of a large number of progenitors and precursors, as well as mature cells, from a small number of undifferentiated (immature) stem cells (SCs), *in vivo* or *ex vivo* [1–3]. Generally, SCs guarantee steady-state homeostasis in every tissue-generating system. It has been believed for a long time that only embryonic SCs (ESCs) are totipotent/pluripotent, since SC plasticity is essential in early development. Thus, for a long time, cellular totipotency/pluripotency was considered a property of only specific ESCs. However, the most primitive adult SC compartments also have comparatively 'limitless' self-renewal potential, as well as the ability to 'switch' into other cell lineages (inter-systemic SC plasticity) [2–6].

During cytopoiesis, a complex system of interactive substances, cytokines, growth factors, and inhibitors is regulated and balanced. A partial or complete loss of balanced control can lead to uncontrolled cell growth or death. Over time, i.e., with ageing, SC activity leads to gradual deterioration of the regenerative potential of cells and tissues and the decline of organ repair/renewal capacity, manifesting as disease and tissue defects, including cancer [2–4].

Hematopoiesis is a dynamic hemobiological process, wherein a large quantity of all the types of blood cells is produced from very primitive hematopoietic SCs (HSCs). HCSs are distributed to different hematopoietic compartments throughout the body, during fetal development and adult life. The bone marrow (BM) of adults, as a primary location, has a high potential of differentiation into pluripotent and committed progenitors, which finally transform into various mature blood cells, necessary for blood turnover and the fight against infections [7–15].

Intrinsic genetic pathways control hematopoiesis. Interactive extrinsic signals from the extracellular matrix (ECM) and other signaling molecular pathways, as well as the stromal cell microenvironment are regulated by basic genetic pathways [2,11–14]. The role of stromal cells, including macrophages, fibroblasts, dendritic, endothelial, and other cells, is to stimulate SCs. Namely, these stromal cells stimulate SCs by producing specific growth factors, such as the Flt3-ligand, c-kit-ligand or SC-factor, as well as interleukins, granulocyte-macrophage colony-stimulating factor and granulocyte colony-stimulating factor (GM-CSF and G-CSF). In addition to secreting cytokines, stromal cells regulate the adhesion of SC molecules and allow them to stay in the BM or migrate to the area where the respective cell type is needed [14–19].

Populations of HSCs, also named $CD34^+$ cells, express the CD34 antigen. Namely, CD34 is the name

Populacije MĆH, označene i kao *CD34*$^+$ ćelije, iskazuju (ekspresija) antigen *CD34*. Naime, *CD34* je ime koje je dato transmembranskom glikoproteinu, prisutnom na površini MĆH, ali i nekih stromalnih ćelija. Adultne ćelije koje eksprimuju *CD34* antigena, a koje su poreklom iz kostne srži ili periferne krvi, omogućavaju kompletnu i dugotrajnu repopulaciju kostne srži primaoca (nakon prihvatanja kalema), sa sledstvenom rekonstitucijom hematopoeze [16–21].

Po pravilu, transplantacija MĆ uključuje intenzivnu radiohemoterapiju (mijeloablacija) uz infuziju prikupljenih ćelija, što se sprovodi da bi se postigla eliminacija osnovne bolesti i/ili eliminisali određeni poremećaji, kao i da bi se poboljšalo kliničko stanje bolesnika. Kod bolesnika, kod kojih ne može biti primenjena visokodozna radiohemoterapija zbog starosti ili komorbiditeta, može biti sprovedena procedura kondicioniranja smanjenog intenziteta (engl. *reduced-intensity conditioning – RIC*) [1–3,8,9]. Kako bi mogle da se daju bolesniku odmah nakon prikupljanja ili nakon dugoročnog čuvanja u zamrznutom stanju – krioprezervacija [2–5], MĆ se mogu prikupiti na tri načina [8,17–22]:

a) višestrukom aspiracijom iz kostne srži;
b) prikupljanjem iz periferne krvi (engl. *peripheral blood – PB*) nakon mobilizacije hemoterapijom i/ili faktorima rasta (rHuG-CSF);
c) izdvajanjem iz krvi pupčane vrpce (engl. *umbilical cord blood – UCB*).

Hematološka oboljenja (uglavnom maligna) kao i određena benigna oboljenja (teška kombinovana imunodeficijencija – TKI, teška aplastična anemija i različita autoimunska ili metabolička oboljenja) do sada su bila najčešća indikacija za transplantaciju MĆ. U današnje vreme, transplantati MĆ prikupljeni iz periferne krvi ili kostne srži su češći kod adultne alogene ili autologne transplantacije. Transplantati MĆ izdvojeni iz krvi pupčane vrpce pokazali su ohrabrujuće rezultate u pedijatriji, uglavnom u slučajevima kada nije moguće naći podudarnog nesrodnog davaoca MĆ [2–4,8].

Plastičnost MĆ je fenomen intersistemske ćelijske plastičnosti, koji odslikava široki fenotipski potencijal vrlo primitivnih MĆ, sposobnih za ukalemljenje u različita tkiva tokom implantacije autolognih MĆ na oštećeno mesto, uz sledstveno transdiferentovanje u ćelijske linije tkiva/organa domaćina i razvoj terapeutske mikroangiogeneze (neovaskularizacije) podstaknute faktorima angiogeneze [11–15,23–25].

Intenziviranjem transplantacija MĆ i uvođenjem inovativnih oblika regenerativne i restorativne ćelijske terapije, došlo je do povećanja potreba za MĆ kao i za praktičnim operativnim procedurama i metodama manipulacije ovih ćelija. Stoga, ovaj rad sažima hemobiologiju MĆ i praktične aspekte optimizacije prikupljanja, prečišćavanja i kliničke primene MĆ.

given to a transmembrane glycoprotein present on the surface of HSCs and some stromal cells. Adult cells expressing the CD34 antigen, obtained from BM or peripheral blood (PB), stimulate complete and long-term repopulation of the recipient's bone marrow (following engraftment), with subsequent hematopoietic reconstitution [16–21].

Traditionally, SC transplantation involves intensive radiochemotherapy (myeloablation) coupled with infusion of collected cells, which is done in order to achieve the eradication of the underlying disease and/or eliminate certain disorders, as well as improve the clinical status of the patient. Patients who are ineligible for high-dose radio-chemotherapy because of their age or comorbidity, may be offered a similar procedure with reduced-intensity conditioning (RIC) [1–3,8,9]. In order to be administered immediately after harvesting or after long-term storage in the frozen state – cryopreservation [2–5], SCs can be collected in three ways [8,17–22]:

a) multiple aspirations from BM;
b) harvesting from PB after mobilization with chemotherapy and/or growth factors (rHuG-CSF);
c) processing from umbilical cord blood (UCB).

Hematological diseases (mostly malignant) and certain benign diseases (severe combined immunodeficiency – SCID, severe aplastic anemia, and different autoimmune or metabolic disorders) have thus far been the most common indication for SC transplantation. Nowadays, SC transplants derived from PB and BM are more common in adult allogeneic or autologous transplantation. UCB-derived SC transplantations have achieved encouraging results in the pediatric setting, mainly when a matched unrelated SC donor is not available [2–4,8].

SC plasticity is a phenomenon of inter-systemic cell plasticity, which reflects the wide-ranging phenotypic potential of very primitive SCs, capable of homing to different tissues during the implantation of autologous SCs into the damaged area, with subsequent transdifferentiation into cell lineages of the host tissue/organ and the development of therapeutic micro-angiogenesis i.e., neovascularization stimulated by angiogenesis growth factors [11–15,23–25].

Both the intensification of SC transplantations and the introduction of innovative cell-mediated restorative or regenerative therapy, increase the need for SCs and for practical operating procedures and manipulation methods in relation to these cells. Therefore, this paper summarizes the SC hemobiology and the practical aspects of the optimization of cell harvesting, purification, and clinical use.

EMBRIONALNI NASPRAM ADULTNIH ODELJAKA MATIČNIH ĆELIJA

Odeljci MĆ uključuju populacije ćelija koje su karakteristične i za EMĆ i za adultno tkivo. Opšte uzev, samo su EMĆ ćelije „prave" totipotentne/pluripotentne ćelije, zato što je fenomen intersistemske plastičnosti MĆ tokom ranog razvoja živih bića od ključnog značaja. U pitanju je sposobnost EMĆ da se razviju u svaku od tri vrste embrionalnog tkiva. Nasuprot tome, smatra se da su MĆ kod odraslih osoba po pravilu ograničene, i u regenerativnom potencijalu i u potencijalu diferentovanja, na tkiva koja „nastanjuju" [6]. Stoga, hepatociti mogu da proliferiraju (i manje-više da se diferentuju) nakon delimične hepatektomije; MĆH mogu da rekonstituišu ćelije krvi nakon oštećenja kostne srži (usled hemoterapije i/ili zračenja); progenitori keratinocita mogu da učestvuju u zarastanju rana; a određene „satelitske" ćelije" mogu da obnove oštećene skeletne mišiće. Uz ulogu u regeneraciji oštećenih tkiva, MĆ imaju važnu funkciju u održavanju homeostaze tkiva. Na primer, MĆ imaju specifičnu ulogu u održavanju tkivne homeostaze tokom celog života jedinke [2–6].

Embrionalne matične ćelije

Kao što je već navedeno, samo su EMĆ validne odnosno „autentične" totipotentne/pluripotentne MĆ, zbog njihovog potencijala da se diferentuju u bilo koju od ćelijskih linija, odnosno njihove sposobnosti da sazru u bilo koji od tri tipa tkiva/germinativnih slojeva u ljudskom organizmu – endoderm, ektoderm ili mezoderm. Nasuprot tome, smatra se da su MĆ kod odraslih osoba, po pravilu, ograničene u svom razvojnom i regenerativnom potencijalu na tkiva koja „nastanjuju", uprkos određenim nalazima u današnje vreme koji ukazuju na to da neke adultne MĆ pokazuju plastičnost koja je slična biološkom potencijalu EMĆ. Naime, vrlo primitivne ćelije potekle iz kostne srži, kao što su veoma male MĆ nalik embrionalnim (engl. *very small embryonic-like SCs – VSELs*), takođe imaju sposobnost da se razviju u razne somatske ćelije, zahvaljujući već spomenutoj plastičnosti MĆ kojom se odlikuju [2,26–34].

Doba inovacija u oblasti fiziologije embrionalnih ćelija je započelo krajem prošlog veka izdvajanjem ćelija koje poseduju totipotentnost (potencijal da se diferentuju u bilo koju od ćelijskih linija u ljudskom telu), iz humanih blastocisti i fetalnog tkiva. Nakon toga, naučnici su opisali važne hemobiološke i molekularne karakteristike ovih ćelija i unapredili metode njihove kultivacije [2–6].

Usled najvišeg stepena ćelijske plastičnosti, zigot se smatra „autentičnom" totipotentnom/pluripotentnom MĆ. Do kraja petog ili šestog dana deobe, nadalje

EMBRYONIC VERSUS ADULT STEM CELL COMPARTMENTS

SC compartments include cell populations characteristic of both ESCs and adult tissue. Generally, only ESCs are 'true' totipotent/pluripotent cells, because the phenomenon of inter-systemic SC plasticity during early development of living organisms is critical. It is the ability of ESCs to develop into all three embryonic tissue types. Conversely, SCs in adults are characteristically considered to be restricted, in their regenerative as well as their differentiative potential, to tissues where they reside [6]. Therefore, hepatocytes can proliferate (and more or less differentiate) following partial hepatectomy; HSCs can reconstitute blood cells after BM damage (due to chemotherapy and/or irradiation); keratinocyte progenitors can participate in wound healing; and certain 'satellite' cells can repair injured skeletal muscles. In addition to their role in the regeneration of damaged tissues, SCs have an important function in maintaining tissue homeostasis. For example, SCs have a specific role in maintaining tissue homeostasis throughout the entire life of an individual [2–6].

Embryonic stem cells

As stated above, only ESCs are valid or 'authentic' totipotent/pluripotent SCs, due to their potential to differentiate into any of the cell lineages, i.e., their ability to mature into any of the three tissue types/germ layers in the human body – the endoderm, ectoderm, or mesoderm. Conversely, SCs in adults are considered limited in their developmental and regenerative potential, typically to the tissues where they reside, despite the current findings that some adult SCs have SC plasticity, similar to the biological potential of ESCs. Namely, very primitive BM-derived cells, such as very small embryonic-like SCs (VSELs), are also able to develop into a variety of somatic cells, due to the aforementioned SC plasticity [2,26–34].

The age of innovation in ESC physiology began at the end of the last century, with the separation of cells, which possess totipotency (the potential to differentiate into any of the cell lineages in the human body), from human blastocysts and fetal tissue. Subsequently, researchers described the important hemobiological and molecular characteristics of these cells and improved methods for their cultivation [2–6].

Due to the highest degree of cell plasticity, the zygote is considered to be the 'authentic' totipotent/pluripotent SC. By the end of the fifth or sixth day of division, it further develops from the older cells of the blastocyte that initiate the expansion of the coding sequence for specific functions, which makes it possible

se razvija iz starih ćelija blastocite koje podstiču širenje kodne sekvence za specifične funkcije, čime postaje moguće da se izoluju EMĆ. Akumulacija fetalnih MĆ u fetalnoj jetri čini ove ćelije hipotetički pogodnim za ekstrakciju iz balstocite i kultivaciju ex vivo u MĆ. Posle njihove transplantacije bolesnicima, smanjena je verovatnoća odbacivanja alotransplantata, pošto ove MĆ imaju vrlo malo ili nimalo proteina okidača imunskog sistema na svojoj površini [2,3,5].

Uprkos jakoj etičkoj argumentaciji da se istraživanje EMĆ, a naročito njihova potencijalna klinička primena, graniče sa ubistvom iz nehata [3,5], EMĆ najviše obećavaju kao ćelije koje se mogu potencijalno presaditi, od svih ostalih MĆ. Naime, bazična istraživanja i neka pretklinička ispitivanja EMĆ su se pokazala kao značajna u razvoju novih metoda zamene ćelija i strategija za regenerisanje oštećenih tkiva i ponovno uspostavljanje ključnih funkcija u obolelom ljudskom organizmu [2,26–34].

Pojam i funkcionalnost adultnih matičnih ćelija

Adultne MĆ identifikovane u raznim tkivima/organima (kostna srž, periferna krv, zubna pulpa, masno tkivo, fetalna jetra, krvni sudovi, mozak, srce, skeletni mišići, koža, gušterača, i gastrointestinalni trakt) obično su zrelije od EMĆ [2–6]. Ranije se smatralo da su adultne MĆ „nesposobne" da proizvedu ćelijske linije tri već navedena tipa tkiva (endoderm, ektoderm i mezoderm) zato što ne mogu da se „podmlade" (u ranijoj ćelijskoj fazi) procesom diferentovanja ćelija – uz proces transdiferentovanja. Adultne MĆ su sposobne da se samoobnavljaju, ali se češće dele kako bi proizvele progenitore i prekursore, kao i zrele ćelije specifičnih ćelijskih linija.

Slika 1 pokazuje da samo kombinacija nekoliko uslova može da posluži kao potvrda da su se neke MĆ, dobijene iz kostne srži, zaista transformisale u somatske ćelije (ćelije specifične za solidne organe) [2,4,23–25].

MĆH su nesumnjivo najbolje proučeni specifični tip MĆ kod odraslih osoba koje imaju potencijal da rekonstituišu sve ćelije krvi. Obe klase MĆH, kratkotrajno (engl. *short-term HSCs – ST-HSCs*) i dugotrajno kolonišuće ćelije (engl. *long-term HSCs – LT-HSCs*), mogu da rekonstituišu krv eksperimentalnih životinja – i to, na jedan do dva meseca (*ST-HSCs*), odnosno na više od šest meseci (*LT-HSCs*) [2,5]. Nakon ukalemljenja, MĆ dobijene iz kostne srži mogu da prođu proces koji se sastoji iz više faza, uključujući migraciju, konverziju u novi ćelijski fenotip, kao i ispoljavanje funkcija karakterističnih za tkiva koja „nastanjuju" [4,23,24].

MĆ dobijene iz kostne srži su heterogena populacija sa morfološkim i funkcionalnim karakteristikama tkivno opredeljenih MĆ (engl. *tissue committed SCs – TCSCs*). Moguće je da su zadužene za reparaciju manjih

to isolate ESCs. Accumulation of fetal SCs in the fetal liver makes them hypothetically suitable to be extracted from the blastocyte and to be cultured ex vivo into SCs. After their transplantation into an individual, the possibility of rejection is reduced, since these SCs have little or no immunity-triggering proteins on their cell surface [2,3,5].

Despite strong ethical arguments that ESC research, and especially potential clinical use, border on negligent manslaughter [3,5], ESCs are the most promising potentially transplantable SCs. Namely, basic research and some preclinical investigations with ESCs have proven to be important for the development of new cell-replacement methods and strategies to restore damaged tissues and re-establish critical functions of the diseased human body [2,26–34].

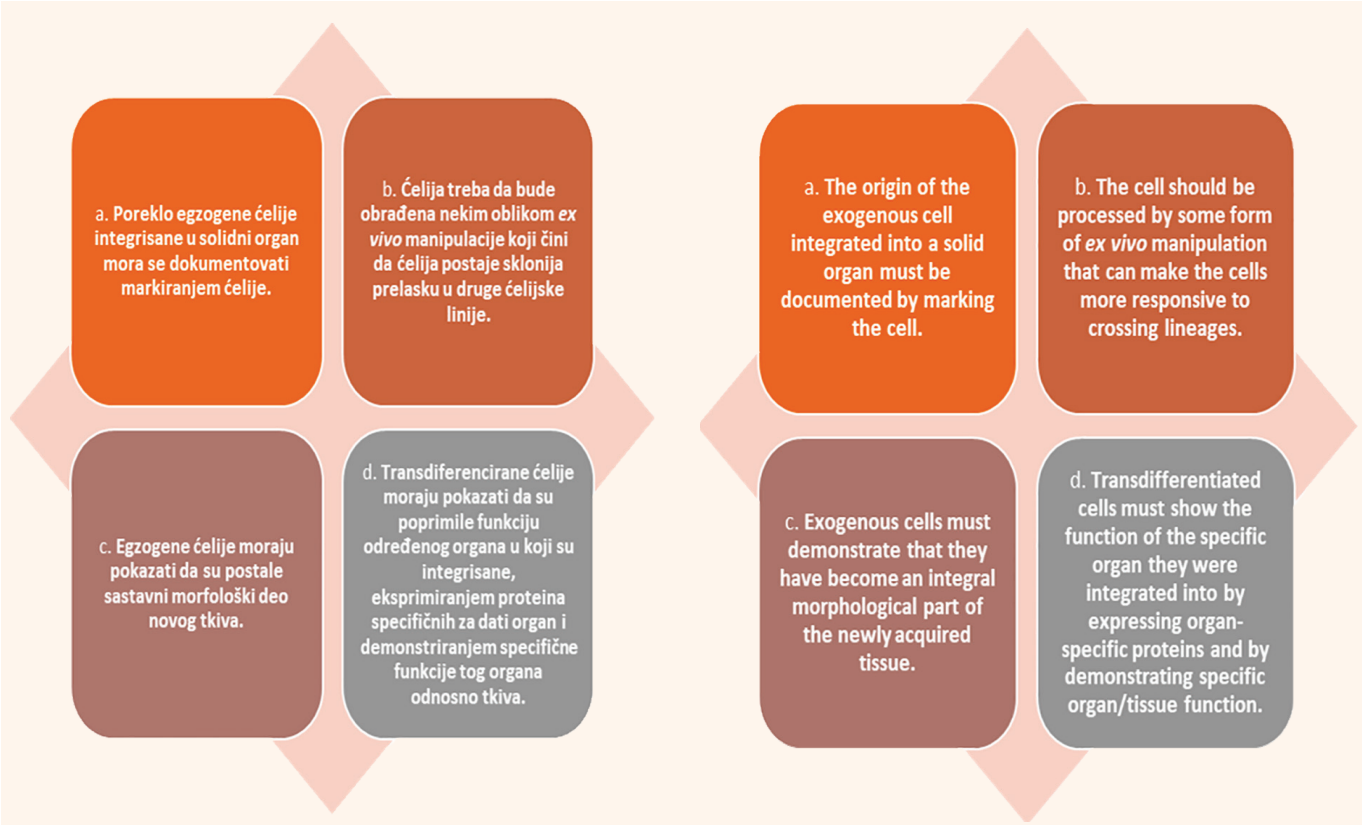
Adult stem cells – concept and functionality

Adult SCs identified in various tissues/organs (BM, PB, dental pulp, adipose tissue, fetal liver, blood vessels, brain, heart, skeletal muscle, skin, pancreas, and the gastrointestinal tract) are usually more mature than ESCs [2–6]. Previously, adult SCs were considered 'incapable' of producing cell lineages of the three above-mentioned tissue types (endoderm, ectoderm and mesoderm) because they could not be 'rejuvenated' (in the earlier cell phase) by the process of cell dedifferentiation – accompanied by transdifferentiation. Adult SCs are capable of self-renewal, but divide more frequently to produce progenitors and precursors, as well as mature cells of specific cell lineages.

Figure 1 shows that only the combination of several conditions can be used to confirm that some SCs, derived from BM, have in fact transformed into somatic cells (cells specific to solid organs) [2,4,23–25].

HSCs are undoubtedly the most thoroughly understood specific type of SCs in adults which have the potential to reconstitute all blood cells. Both identified classes of HSCs, short-term HSCs (ST-HSCs) and long-term HSCs (LT-HSCs), can reconstitute the blood of experimental animals for one to two months, and more than 6 months, respectively [2,5]. After homing, BM-derived SCs can undergo a multistep process involving migration, conversion to a new cellular phenotype, and expression of functions characteristic of the tissue where they reside [4,23,24].

BM-derived SCs are a heterogeneous population of cells with morphological and functional characteristics of tissue committed SCs (TCSCs). They may be in charge of healing minor tissue damage; their number among mononuclear cells (MNCs) is very low – approximately one cell per 1,000 to 10,000 total nucleated



Slika 1. Pokazuje da samo kombinacija nekoliko uslova može da posluži kao potvrda da su se neke MĆ, dobijene iz kostne srži, zaista transformisale u somatske ćelije (ćelije specifične za solidne organe) [2,4,23–25]

Figure 1. Shows that only the combination of several conditions can be used to confirm that some SCs, derived from BM, have in fact transformed into somatic cells (cells specific to solid organs) [2,4,23–25]

oštećenja tkiva; njihov broj među mononuklearnim ćelijama (engl. *mononuclear cells – MNCs*) je veoma nizak – oko jedna ćelija na 1.000 do 10.000 od ukupnog broj ćelija s jedrom (engl. *total nucleated cells – TNCs*) u kostnoj srži [2,19]. Međutim, kod teških oštećenja (infarkt miokarda ili moždani udar), oni imaju mogućnost da ispolje svoj puni terapijski potencijal. Migracija ovih ćelija u oštećene zone zavisi od „signala ukalemljenja", koji može biti neefikasan u prisustvu citokina ili proteoliznih enzima, koje oslobađaju leukociti i/ili makrofagi oštećenog tkiva [23]. Ipak, dok su „zarobljene" odnosno „inkapsulirane" u kostnoj srži, ove ćelije mogu biti u posebnom „latentnom stadijumu" – nisu u potpunosti funkcionalne, te su im potrebni odgovarajući aktivacioni signali [2–4,23].

Mezenhimske matične ćelije/stromalne ćelije

U poređenju sa EMĆ i indukovanim pluripotentnim MĆ (engl. *induced pluripotent SCs – iPSCs*), mezenhimske/stromalne MĆ (engl. *mesenchymal stem/stromal cells – MSCs*) imaju terapijski potencijal i bezbednosni profil visokog kvaliteta [2–6]. Molekularne i funkcionalne karakteristike zubne pulpe čine je važnim izvorom MĆ, uključujući tu i adultne mezenhimske MĆ [2,34–38]. Smatra se da su mezenhimske MĆ ektomezenhimskog

cells (TNCs) in BM [2,19]. However, in severe injuries (heart infarction or stroke), they have the possibility to express their full therapeutic potential. The migration of these cells to the damaged areas depends on the 'homing signal', which might be inefficient in the presence of cytokines or proteolytic enzymes released from injured leukocytes and/or macrophages [23]. Nonetheless, these cells while 'captured' or 'encapsulated' in BM might be in a particular 'latent stage' – not fully functional, and in need of the appropriate activation signals [2–4,23].

Mesenchymal stem cells/stromal cells

Compared to ESCs and induced pluripotent SCs (iPSCs), mesenchymal stem cells/stromal cells (MSCs) have a high-quality therapeutic potential and safety profile [2–6]. The molecular and functional characteristics of the dental pulp make it an important source of dental pulp SCs, including adult MSCs [2,34–38]. MSCs of ectomesenchymal origin located in the perivascular niche are considered highly proliferative, multipotent, and similar to BM-derived SCs. Other dental pulp SCs (DPSCs), stem cells from human exfoliated deciduous teeth (SHED), and immature dental pulp cells (IDPC) can transdifferentiate into various cells, such as

porekla, koje se nalaze u perivaskularnoj niši, visoko proliferativne, multipotentne i slične MĆ poteklim iz kostne srži. Druge MĆ iz zubne pulpe (engl. *dental pulp SCs – DPSCs*), MĆ iz „eksfoliranih" humanih mlečnih zuba (engl. *stem cells from human exfoliated deciduous teeth – SHED*), kao i primitivne ćelije zubne pulpe (engl. *immature dental pulp cells – IDPC*) mogu se transdiferentovati u različite ćelije, kao što su odontoblasti, hondrociti, osteoblasti, adipociti, nervne/glijalne ćelije, ćelije glatkih mišića, ćelije skeletnih mišića, i druge ćelije. Buduća istraživanja trebalo bi da pruže, uz podatke o MĆ zubne pulpe, kompleksne podatke i o mnogim drugim humanim tkivima kao potencijalnim izvorima MĆ odgovornih za razvoj/regeneraciju tkiva.

Pojam veoma malih matičnih ćelija nalik embrionalnim (VSEL ćelije)

Specifični tip ćelija, koji je prvobitno izolovan iz kostne srži, liči na EMĆ i u stanju je da imitira njihovu sposobnost transdiferentovanja u druge tipove ćelija. Štaviše, ove ćelije mogu da se transdiferentuju u nove ćelijske linije iz više od jednog germinativnog sloja – u nervne, ćelije srca, gušterače, kao i ćelije drugih tkiva odnosno organa. Retajčak i saradnici su bili prvi koji su identifikovali *VSEL* ćelije, poseban tip ćelija koji se ponaša drugačije od ostalih MĆ dobijenih iz adultne kostne srži [26–30]. Iako su *VSEL* ćelije u suštini vrlo slične ili iste, po ultrastrukturi i proteinskim markerima, kao i EMĆ [2,27–29], one se smatraju „kontaminantima", koji zapravo doprinose pozitivnom regenerativnom kliničkom ishodu [26–32]. Najzad, postoje podaci koji potvrđuju da VSEL ćelije regenerišu, dok mezenhimske MĆ podmlađuju, obolela reproduktivna tkiva [33]. Međutim, autori ovakvih studija su izneli pretpostavku da implantacija *MSC* ćelija naprosto oslobađa faktore rasta ili specifične citokine koji su od ključnog značaja u procesu diferentovanja MĆ „nastanjenih" u tkivu, u spermatozoide ili jajne ćelije [33]. Ovo je zanimljiv koncept u regenerativnoj medicini koji bi trebalo da se ozbiljno razmotri u budućim istraživanjima i pretkliničkim ispitivanjima na ljudima.

Pojam i primena indukovanih pluripotentnih matičnih ćelija (iPSC ćelije)

Izuzetan doprinos istraživanjima i primeni MĆ dali su Džon Gurdon i Šinja Jamanaka svojim radom nagrađenim Nobelovom nagradom, 2012. godine, koji je pokazao da, uz primenu ključnih molekula, zrele adultne ćelije/fibroblasti mogu da se diferentuju u pluripotentne MĆ i da se transdiferentuju, odnosno konvertuju u različite zrele ćelijske linije (npr. ćelije srčanog mišića) [39]. Iako su ograničene u pluripotentnosti i u stanju da indukuju maligne ćelije, ove *iPSC* ćelije predstavljaju

odontoblasts, chondrocytes, osteoblasts, adipocytes, neuron/glial cells, smooth muscle cells, skeletal muscle cells, and other cells. Future research should provide us with complex data on many human tissues, in addition to DPSCs, as potential sources of SCs responsible for tissue development/regeneration.

The concept of very small embryonic-like stem cells (VSELs)

A specific type of cells, isolated initially from BM, look like ESCs and appear to mimic their ability to transdifferentiate into other cell types. Furthermore, these cells can transdifferentiate into novel cell lineages from more than one germ layer – into nerve, heart, pancreatic and cells of other tissues/organs. Ratajczak et al. were the first to identify VSELs, a special type of SC that acts differently than other SCs derived from adult BM [26–30]. Although VSELs have basically very similar or the same ultrastructure and protein markers as ESCs [2,27–29], they are considered 'contaminants', which, in fact, contribute to a positive regenerative clinical outcome [26–32]. Finally, there are data confirming that VSELs regenerate, whereas MSCs rejuvenate diseased reproductive tissues [33]. However, the authors of such studies supposed that MSC implantation may simply release growth factors or specific cytokines critical for tissue-resident SCs to differentiate into sperm cells or ova [33]. This is an interesting concept in regenerative medicine, which should be seriously considered in future research and preclinical studies on humans.

The concept and application of induced pluripotent stem cells (iPSCs)

An incredible contribution to SC research and application came from the Nobel prize awarded work of John Gurdon and ShinyaYamanaka (2012), which has shown that, with the use of crucial molecules, mature adult cells/fibroblasts can dedifferentiate into pluripotent SCs and transdifferentiate or convert into different mature cellular lines (e.g., cardiac muscle cells) [39]. Although limited in pluripotency and capable of inducing malignant cells, these iPSCs are a potential source with which clinicians can circumvent certain source-related problems. The future will show their implications and impact on SC therapy.

STEM CELL CATEGORY AND INFLUENCE

The traditional primary cell source for SC transplantation is the bone marrow. Approximately 1 – 3% of TNCs in BM express the CD34 antigen. The $CD34^+$ cells have been found in PB, but in an extremely small number in steady-state hematopoiesis – only 0.01 – 0.05% of TNCs [2,19].

potencijalni izvor MĆ koji može pomoći kliničarima da prevaziđu određene probleme u njihovom obezbeđivanju. Budućnost će pokazati implikacije koje su skopčane sa *iPSC* ćelijama, kao i njihov uticaj na oblast lečenja MĆ.

KATEGORIJA I UTICAJ MATIČNIH ĆELIJA

Tradicionalno, primarni izvor ćelija za transplantaciju MĆ jeste kostna srž. Oko 1 – 3% od ukupnog broja ćelija sa jedrom (*TNC*) u kostnoj srži eksprimira *CD34* antigen. *CD34*$^+$ ćelije su pronađene u kostnoj srži, ali u jako malom broju u stanju stabilne hematopoeze – svega 0,01 – 0,05% od ukupnog broja ćelija sa jedrom [2,19].

Za transplantaciju je od ključnog značaja količina MĆ. Različite populacije *TNC*-a i *MNC*-a (uključujući i MĆ) mogu se dobiti višestrukim (ponovljenim) aspiracijama iz kostne srži, prikupljanjem iz periferne krvi nakon mobilizacije (hemoterapija i/ili *rHuG-CSF*), ili obradom iz krvi pupčane vrpce. Ove ćelije se mogu klinički primeniti (može se izvršiti njihova transplantacija) odmah nakon prikupljanja odnosno krioprezervacije [1–4,10].

Kada se davalac anestezira, ćelije se mogu prikupiti višestrukim aspiracijama iz zadnjih grebena bedrene kosti (ređe prednjih grebena), primenom maksimalnog ciljnog volumena aspirirane kostne srži do 15 ml/kg telesne mase (engl. *kg of body mass – kgbm*) davaoca. Aspirat iz kostne srži mora se filtrirati da bi se odstranile partikule kosti ili lipida kao i agregati ćelija. Rastvor citrata i heparin razblažen u fiziološkom rastvoru (5.000 IU/500 ml) koriste se kao antikoagulansi [2–5].

Kod ABO nekompatibilnih transplantacija MĆ, potrebna je redukcija broja crvenih krvnih zrnaca i količine plazme (obradom aspirata). Redukcija T ćelija se postiže *ex vivo* prečišćavanjem (pozitivna ili negativna imuno-magnetna selekcija ćelija). Procedure obrade i prečišćavanja MĆ omogućavaju redukciju ukupnog broja crvenih krvnih zrnaca za 90%, kao i redukciju T ćelija za 3 – 4 Log_{10} [2–5].

U današnje vreme, broj bolesnika lečenih MĆ dobijenim iz kostne srži neprestano raste, a MĆ dobijene iz kostne srži se koriste za oko 80% alogenih i za praktično sve autologne transplantacije. Karakteristike transplantacija MĆ su sledeće [2–5,19]:

a) minimalno invazivni metod prikupljanja ćelija i odsustvo rizika opšte anestezije;
b) mali volumen afereznog produkta (250 ml) sa većom količinom prikupljenih CD34$^+$ ćelija;
c) visoka stopa prihvatanja kalema (brza rekonstitucija hematopoeze) i nizak nivo morbiditeta uzrokovanog transplantacijom (engl. *transplant-related morbidity – TRM*).

Kada je u pitanju planiranje vremena prikupljanja MĆ, ukupan broj *CD34*$^+$ ćelija u cirkulaciji je maksimalan

For transplantation, the SC quantity is a critical issue. Different populations of TNCs and MNCs (including SCs) can be obtained by multiple aspirations from BM, harvesting from PB after mobilization (chemotherapy and/or rHuG-CSF), and processing from UCB. These cells can be clinically applied (transplanted) immediately after collection or cryopreservation [1–4,10].

When the donor is anesthetized, cells can be collected by multiple aspirations from the posterior (seldom anterior) iliac crests, using the maximal target aspirate volume of up to 15 ml/kg of body mass (kgbm) of the donor. Marrow aspirate must be filtered to remove bone or lipid particles and cell aggregates. Citrate solution and heparin diluted in saline (5,000 IU/500 ml) are used for anticoagulation [2–5].

For ABO incompatible SC transplantations, red blood cell (RBC) count and plasma quantity reduction is required (by aspirate processing). Depletion of T-cells is achieved by *ex vivo* purging (positive or negative immune-magnetic cell selection). Procedures of processing and purging SCs enable the reduction of RBC total quantity by 90% as well as T-cell depletion by 3 – 4 Log_{10} [2–5].

Nowadays, the number of patients treated with PB-derived SCs is constantly growing, and PB-derived SCs are used for about 80% of allogeneic and practically for all autologous transplants. The characteristics of SC transplantations are the following [2–5,19]:

a) minimally invasive cell harvesting method and absence of general anesthesia risks;
b) small harvesting volume (250 ml) with a better yield of CD34$^+$;
c) high engraftment rate (rapid hematopoietic reconstruction) and low transplant-related morbidity (TRM).

Regarding the timing of stem cell harvesting, the CD34$^+$ cell count in the circulation is maximal, i.e., it peaks on the 5th day of rHuG-CSF administration (5 – 10 μg/kgbm per day for allogeneic donors). However, the optimal timing of autologous SC harvesting is more complex, and these patients receive a higher rHuG-CSF dose (12 – 16 μg/kgbm or more, daily) combined with chemotherapy [2–5]. It is important that the count of circulating CD34$^+$ cells should correlate with a high CD34$^+$ yield in the graft. It is estimated that when the number of CD34$^+$ cells in PB is higher than 40/μl, the possibility of obtaining CD34$^+$ ≥ 2.5x10^6 per kgbm in the recipient is 60%, after performing one large-volume leukapheresis (LVL) [2–5,21].

An innovative harvesting protocol uses plerixafor (mozobil) to obtain a sufficient SC quantity from 'poor-mobilizers'. The stromal derived factor 1 (SDF-1) retains SCs in the BM due to its physiological interaction

petog dana nakon primene *rHuG-CSF (5 – 10 μg/kgbm*, na dan, za alogene davaoce). Međutim, planiranje optimalnog vremena autolognog prikupljanja MĆ je ipak kompleksnije, te ovi bolesnici primaju višu dozu *rHuG-CSF (12 – 16 μg/kgbm* ili više, na dan) u kombinaciji sa hemoterapijom [2–5]. Važno je da ukupan broj *CD34*$^+$ ćelija u cirkulaciji korelira sa visokim brojem prikupljenih *CD34*$^+$ ćelija u graftu. Procenjuje se da, kada je broj *CD34*$^+$ ćelija u kostnoj srži viši od 40/μl, mogućnost dobijanja *CD34*$^+$≥ *2,5x10*^{6} po *kgbm* kod primaoca iznosi 60%, nakon leukapareze velikog volumena (engl. *large-volume leukapheresis – LVL*) [2–5,21].

Jedan inovativni protokol prikupljanja ćelija podrazumeva upotrebu pleriksafora (mozobila) kako bi se dobila dovoljna količina MĆ od „loših mobilizera". Faktor poreklom iz strome 1 (engl. *stromal derived factor 1 – SDF-1*), zadržava MĆ u kostnoj srži usled svoje fiziološke interakcije sa hemokinskim receptorom *CXCR*. Pleriksafor je potentan antagonist alfa hemokinskog receptora *CXCR4*, jer uspešno inhibira interakciju *CXCR4 – SDF-1*. Postoje podaci o unapređenom režimu mobilizacije koji primenjuje pleriksafor sa *rHuG-CSF*, zbog većeg broja nezrelih MĆ u cirkulaciji i većeg broja prikupljenih *CD34*$^+$ ćelija u graftu [2–4,20]. Uspešna transplantacija se može očekivati kada se postigne cilj: *CD34*$^+$ ćelije ≥*2–4x10*^{6}*/kgbm* kod primaoca. Naša pretklinička istraživanja su potvrdila da relativna učestalost jedne vrlo primitivne podgrupe MĆ (*CD34*$^+$*/CD90*$^+$) u cirkulaciji može biti praktičan i potencijalno objektivan prediktivni faktor mobilizacije kod planiranja optimalnog vremena za prikupljanje MĆ, kao i prediktor kvaliteta prikupljenog uzorka [1–5,20].

Mogućnost pronalaženja odraslog alogenog davaoca ≤ 30% (za srodne davaoce) i ≤ 85% (za nesrodne davaoce), ukazuje na ograničenu dostupnost davaoca [2–5,18]. Tokom proteklih decenija, krv iz pupčane vrpce korišćena je kao održivi alternativni izvor [1]*HLA*-tipiziranih (podudarnih) MĆ za alogenu transplantaciju [2–5,17].

Neonatalne MĆ dobijaju se iz krvi pupčane vrpce, odmah nakon porođaja, bezbolnom i neinvazivnom metodom prikupljanja. Ove ćelije su manje zrele nego ćelije iz kostne srži. „Naivna" priroda limfocita iz krvi pupčane vrpce omogućava upotrebu *HLA*-delimično podudarnih graftova MĆ, bez povećanog rizika teškog oblika bolesti kalema protiv domaćina (engl. *graft versus host disease – GvHD*), u poređenju sa transplantacijom iz kostne srži dobijene od potpuno podudarnog nesrodnog davaoca [2–5,17]. Zbog ograničenog broja MĆ, krv pupčane vrpce je izvor koji je prihvatljiv za pedijatrijske bolesnike kao i bolesnike kod kojih nije moguće obezbediti podudarnog nesrodnog davaoca.

1 engl. *HLA – human leukocyte antigens*

with chemokine receptor CXCR. Plerixafor is a potent antagonist of the alpha chemokine receptor CXCR4 as it effectively inhibits CXCR4 – SDF-1 interaction. There are data on an improved mobilizing regimen which uses plerixafor with rHuG-CSF on account of a higher immature SC count in the circulation and a high CD34$^+$ yield in the graft [2–4,20]. A successful transplantation can be expected when the target: CD34$^+$ cells ≥ 2–4x10^6/kgbm of the recipient, is achieved. Our preclinical investigation has confirmed that the relative frequency of the very primitive SC subset (CD34$^+$/CD90$^+$) in circulation could be a practical and potentially objective mobilization predictive factor for optimized timing of cell harvesting and a predictor of the harvest quality [1–5,20].

A possibility ≤ 30% (for related donors) and ≤ 85% (for unrelated donors) of finding an adult allogeneic donor, indicates limited donor availability [2–5,18]. In the previous decades, UCB has been used as a viable alternative source of 1HLA-typed (matching) SCs for allogeneic transplantation [2–5,17].

Neonatal SCs are obtained from UCB, immediately after birth, by a painless and non-invasive method of collection. These cells are less mature than those in BM. The 'naïve' nature of UCB lymphocytes allows the use of partially HLA-mismatched SC grafts without an increased risk of severe graft versus host disease (GvHD), as compared to BM transplantation from a completely matched unrelated donor [2–5,17]. Due to a limited number of SCs, the UCB is a cell source accepted for pediatric patients and patients for whom a matched unrelated SC donor is unavailable. Unfortunately, children weighing ≥ 45 kgbm have a higher risk of graft failure [2–5,17].

More recently, SC transplantation from haploidentical donors for the treatment of patients who do not have HLA-matched donors has continued to increase [40,41].

CLINICAL PRACTICE: STEM CELL TRANSPLANTATIONS VERSUS REGENERATIVE MEDICINE

The standard treatment for hematological malignancies and certain benign disorders is the infusion of allogeneic or autologous SCs, collected for the hematopoietic and clinical recovery of patients after high-dose radio-chemotherapy. A similar procedure with RIC may be offered to patients who are ineligible for high dose conditioning due to age or comorbidity. The idea of treating immune-mediated diseases, such as multiple sclerosis and immune-mediated enteropathy, by using SC transplants, is based on the principle that immunoablative treatment can destroy the patient's anti-self lymphocytes [2–5].

1 HLA – human leukocyte antigens

Nažalost, kod dece čija je težina ≥ *45 kgbm* postoji veći rizik da dođe do neprihvatanja kalema [2–5,17].

U novije vreme, u porastu je transplantacija MĆ dobijenih od haploidentičnih davaoca za lečenje bolesnika koji nemaju *HLA*-podudarne donore [40,41].

KLINIČKA PRAKSA: TRANSPLANTACIJE MATIČNIH ĆELIJA NASPRAM REGENERATIVNE MEDICINE

Standardno lečenje hematoloških maligniteta i određenih benignih oboljenja jeste infuzija alogenih ili autolognih MĆ, prikupljenih u cilju hematopoetskog i kliničkog oporavka bolesnika nakon visokodozne radiohemoterapije. Slična procedura – uz primenu *RIC* – se izvodi kod bolesnika kojima nije moguće primeniti visokodozno kondicioniranje, zbog uzrasta ili komorbiditeta. Tretman oboljenja uzrokovanih poremećajem imunskog sistema (kao što su multipla skleroza i autoimunska enteropatija) transplantacijom MĆ, zasnovan je na principu da imunoablativna terapija može uništiti bolesnikove autodestruktivne limfocite [2–5].

Tabela 1 prikazuje najčešće indikacije za transplantaciju MĆ, kao što su hematološka oboljenja i određena oboljenja uzrokovana poremećajem imunskog sistema [1–5,11–15,18–22].

Transplantacije MĆ kod nas su izvedene u lečenju bolesnika sa akutnim leukemijama, hroničnom mijeloidnom leukemijom, multiplim mijelomom, limfomima, kao i bolesnika sa pojedinim karcinomima, ekstragonadalnim neseminomskim tumorom germinativnih ćelija, teškim oblikom aplastične anemije i multiplom sklerozom [2–5,20–22].

Specifični klinički aspekti transplantacije MĆ u lečenju različitih hematoonkoloških i drugih oboljenja, kao što su optimalno vreme transplantacije, terapijska efikasnost i komplikacije, neće biti detaljno opisani i analizirani u ovom radu. Ukratko, efikasnost transplantacije MĆ zavisila je od vrste bolesti, stadijuma bolesti, njene osetljivosti na hemoterapiju, kao i od uzrasta bolesnika, njegovog opšteg zdravstvenog stanja, te stepena podudaranja u sistemu *HLA* [2–4,20–22].

Opšte poznati efekti alogene transplantacije u hematoonkologiji se tek od nedavno koriste za precizno izučavanje sistema koji jasno odvaja pozitivne efekte fenomena kalem protiv leukemije (engl. *graft versus leukemia – GvL*) od negativnih efekata *GvHD*. Uz opšte poznate pozitivne rezultate u lečenju bolesnika sa multiplim mijelomom i leukemijom [2,42], kod nas je ustanovljeno da je infuzija donor specifičnih limfocita (engl. *donor-specific lymphocyte infusion – DLI*) efikasna bila u lečenju bolesnika sa Filadelfija hromozom-pozitivnom hroničnom mijeloidnom leukemijom (engl. *Philadelphia chromosome-positive CML*) (za vreme relapsa nakon

Table 1 presents the most common indications for SC transplantation, such as hematological diseases and certain immune-mediated disorders [1–5,11–15,18–22].

In our Center, we use SC transplantations to treat patients with acute leukemia (lymphoblastic and non-lymphoblastic), chronic myeloid leukemia, multiple myeloma, Hodgkin and non-Hodgkin lymphoma, as well as patients with breast and ovarian cancer, extragonadal non-seminal germ cell tumors, severe aplastic anemia, and severe multiple sclerosis [2–5,20-22].

However, specific clinical aspects of SC transplantation in the treatment of various hemato-oncological and other disorders, such as optimal transplantation

Tabela 1. Uobičajene i relativne indikacije za transplantaciju matičnih ćelija

Table 1. Common and relative indications for SC transplantation

Hematološka oboljenja / *Hematological diseases*
◆ Akutna leukemija / *Acute leukemia*
– Akutna mijeloidna leukemija / *Acute myeloid leukemia*
– Akutna limfoblastna leukemija / *Acute lymphoblastic leukemia*
◆ Mijelodisplazija / *Myelodysplasia*
◆ Mijeloproliferativni poremećaji / *Myeloproliferative disorders*
– Hronična mijeloidna leukemija (retko) / *Chronic myeloid leukemia (seldom)*
– Ostali mijeloproliferativni poremećaji / *Other myeloproliferative disorders*
◆ Hronične limfoproliferativne bolesti / *Chronic lymphoproliferative diseases*
– Hočkinov limfom / *Hodgkin lymphoma*
– Ne-Hočkinov limfom / *Non-Hodgkin lymphoma*
- Visoko agresivni ne-Hočkinov limfom / *High grade non-Hodgkin lymphoma*
- Nisko agresivni ne-Hočkinov limfom / *Low grade non-Hodgkin lymphoma*
– Multipli mijelom / *Multiple myeloma*
◆ Aplastična anemija / *Aplastic anemia*
Ostali poremećaji /*Other disorders*
◆ Teška kombinovana imunodeficijencija / *Severe combined immunodeficiency (SCID)*
◆ Stečene imunodeficijencije / *Acquired immune deficiencies*
◆ Talasemija / *Thalassemia*
◆ Metabolički poremećaji / *Metabolic deficiencies*
◆ Autoimunska oboljenja (nrp. multipla skleroza – MS) / *Autoimmune disorders (e.g., multiple sclerosis – MS)*
◆ **Ne-hematološki maligniteti / *Nonhematological malignancies***
◆ Neuroblastom / *Neuroblastoma*
◆ Vilmsov tumor / *Wilms' tumor*
◆ Rabadomiosarkom / *Rhabdomyosarcoma*
◆ Juingov sarkom / *Ewing sarcoma*
◆ Karcinom dojke, jajnika, testisa / *Breast, ovarian, testicular cancer*

transplantacije MĆ). Uz to, uveden je originalni *in vitro* test, označen kao „Test mešanih progenitora" radi predviđanja kliničkog ishoda terapije primenom *DLI* [2,5,42]. Iako su *TRM* i mortalitet radikalno smanjeni, transplantacije MĆ mogu rezultovati brojnim komplikacijama, uključujući i one najčešće – neuspešno prihvatanje kalema, virusne ili oportunističke infekcije, te akutni ili hronični *GvHD* [1–5].

U oblasti regenerativne medicine, intersistemska plastičnost MĆ opravdava upotrebu primitivnih MĆ u lečenju bolesnika sa oštećenjima srca, jetre, pankreasa i drugih organa ili tkiva. Rezultati kliničkih studija ukazuju na transdiferentovanje MĆ u kardiomiocite nakon primene i ukalemljenja (homing) u oštećenoj, odnosno ishemijskoj zoni miokarda. Kostna srž je najčešće korišćen izvor ćelija koje se koriste za postizanje kliničkog oporavka miokarda zbog sadržaja mešavine različitih populacija progenitora – uključujući MĆH, ali i brojne ne-hematopoetske ćelije (npr. *TCSC*, *MSC* i *VSEL* ćelija), koje mogu da se transdiferentuju u različite ćelijske linije [14,23,43–45].

Naš Centar za regenerativnu medicinu počeo je sa intrakoronarnom aplikacijom (u kardiologiji) i intramiokardnom implantacijom (u kardiohirurgiji) mononukleusnih ćelija (sa prisutnim MĆ – MNĆ/MĆ) 2003, odnosno 2006. godine [14,23–25]. Preliminarni rezultati pokazali su da je lečenje akutnog infarkta miokarda sa ST elevacijom (engl. *ST-elevation myocardial infarction – STEMI*) pomoću intrakoronarne injekcije (petog dana od infarkta) autolognih MNC/SC-a, prikupljenih iz aktivirane kostne srži (kostna srž aktivirana pomoću *G-CSF*-a u jednoj dozi; 3 – 5 μg/*kgbm*), bilo delotvorno i bezbedno. Ejekciona frakcija leve komore (engl. *left ventricular ejection fraction – LVEF*) se popravila (povećanje = 5,5 ± 6,6%; četvoromesečni period praćenja), a veličina infarktne zone (engl. *infarction size – IS*) se smanjila (smanjenje = 6,2 ± 5,0%). Dugoročni pozitivni *LVEF/IS* efekti su bili umereni. Nisu registrovani kardiovaskularni mortalitet, ponovljeni infarkt, klinički manifestna srčana insuficijencija, maligna aritmija ili drugi neželjeni događaji [14,23,24].

Naši prvobitni rezultati u domenu hirurške revaskularizacije srca ili koronarne bajpas hirurgije (engl. *coronary artery bypass grafting – CABG*) udružene intramiokardnom implantacijom MNĆ/MĆ (grupa: *CABG* plus MNĆ/MĆ) pokazali su nedvosmislenu superiornost ove metode. *LVEF* se značajno popravila (povećanje = 5,0 ± 4,2) kod ovih bolesnika. Takođe je potvrđen značajno poboljšani funkcionalni kapacitet ($p < 0,001$) pomoću šestominutnog testa hodanja (engl. *6-minute walk test – 6-MWT*); (6-MWT; šestomesečni period praćenja; $p < 0,001$) u grupi *CABG* plus MNĆ/MĆ naspram kontrolne grupe (samo *CABG*). Ova terapija se takođe pokazala kao bezbedan terapijski pristup

timing, therapeutic efficacy, and complications, are not described and discussed in this paper in detail. In brief, the efficacy of SC transplantations depends on the type of disease, its stage, its sensitivity to chemotherapy, the patient's age, and their general health status, as well as the degree of HLA-matching [2–4,20–22].

The well-known beneficial effects of allogeneic SC transplantation in hemato-oncology have only recently been applied in designing a system that separates the positive effects of graft versus leukemia (GvL) from the negative effects of graft versus host disease (GvHD). In addition to the already known favorable results in the treatment of patients with multiple myeloma and leukemia [2,42], we have also found donor-specific lymphocyte infusion (DLI) to be effective in patients with Philadelphia chromosome-positive CML (relapsed after SC transplantation), and we have introduced an original *in vitro* test, the so called 'Test of Mixed Progenitors', to predict the clinical outcome of DLI treatment [2,5,42]. Although TRM and mortality have been radically reduced, SC transplantations can involve a number of complications, including the most common ones – engrafting failure, viral or opportunistic infections, and acute or chronic GvHD [1–5].

In the field of regenerative medicine, intersystemic SC plasticity provides a rationale for the use of immature SCs in the treatment of patients with damage to the heart, liver, pancreas, and other organs/tissues. Findings from clinical studies indicate transdifferentiation in cardiomyocytes after the administration and homing of SCs in the damaged or ischemic region of the myocardium. BM was the most frequently used source of cells for clinical cardiac repair, due to its complex mixture of progenitors, including HSCs, but also different non-hematopoietic cells (e.g., TCSCs, MSCs and VSELs) which can transdifferentiate into various cell lineages [14,23,43–45].

Our Center of Regenerative Medicine began applying intracoronary injection (cardiology) and intramyocardial implantation (cardiac surgery) of MNC/SCs in 2003 and 2006, respectively [14,23–25]. Preliminary results have shown that treatment of acute ST-elevation myocardial infarction (STEMI) with intracoronary injection (on the 5th day upon infarction) of autologous MNC/SCs, collected from primed/activated BM (BM activated by G-CSF, single dose; 3 – 5 μg/kgbm), was effective and safe. The left ventricular ejection fraction (LVEF) improved (increase = 5.5 ± 6.6%; 4-month follow-up period) and the infarction size (IS) decreased (decrease = 6.2 ± 5.0%). The long-term positive LVEF/IS effects were moderate. No cardiovascular mortality, reinfarction, clinically manifest heart failure, malignant arrhythmia, or other adverse events were detected [14,23,24].

– kardiovaskularni mortalitet je bio značajno niži (petogodišnji period praćenja; $p = 0,049$) kod bolesnika iz grupe *CABG* plus MNĆ/MĆ [2,25,45].

ZAKLJUČAK

Transplantacija MĆ je, bez sumnje, veoma efikasan modalitet lečenja hematoloških maligniteta i određenih bolesti nastalih zbog poremećaja imunskog sistema. Intenziviranje kliničke upotrebe terapije ćelijama (transplantacija MĆ i regenerativna medicina) povećala je potrebu za većim kvantitetom i boljim kvalitetom suspenzije MĆ (veći prinos prikupljenih ćelija i bolja vijabilnost). Unapređeni protokoli prikupljanja, prečišćavanja i kriokonzervacije, kao i poboljšane extrakorporalne operativne procedure, svele su na minimum mehanička i termička oštećenja tokom *ex vivo* manipulacije ćelijama. Neophodno je raspolagati optimizovanim procedurama ekstrakorporalne manipulacije MĆ. Primena koncepta plastičnosti MĆ podstakla je sve širu terapijsku primenu primitivnih MĆ za obnavljanje, odnosno regeneraciju organa. Neophodna su buduća bazična istraživanja, kao i brojne randomizovane kontrolisane velike kliničke studije u oblastima transplantacijske i regenerativne primene MĆ, radi bolje procene efikasnosti ove revolucionarne terapije u lečenju ishemijske bolesti srca, ali i oštećenja drugih tkiva, odnosno organa.

KRATAK PREGLED NAJVAŽNIJIH TEMA

Primena konvencionalnih transplantacija MĆ je, bez sumnje, delotvoran oblik lečenja hematoloških bolesnika. Opšte poznati efekti alogenih transplantacija u hematoonkologiji se koriste kako bi se razradio sistem koji odvaja pozitivne efekte *GvL*, od negativnih efekata *GvHD*.

Plastičnost MĆ je fenomen intersistemske ćelijske plastičnosti, koji omogućava široki fenotipski potencijal vrlo primitivnih MĆ, sposobnih za ukalemljenje (homing) u različita tkiva posle implantacije na oštećeno mesto, uz sledstveno transdiferentovanje u ćelijske linije tkiva/organa domaćina.

Intersistemska plastičnost primitivnih MĆ opravdava njihovu primenu u lečenju bolesnika sa oštećenjima na srcu, u jetri, pankreasu, i drugim organima ili tkivima.

Sukob interesa: Nije prijavljen.

Our initial results in coronary artery bypass grafting (CABG) followed by MNC/SC intramyocardial implantation (the CABG plus MNC/SC group) showed the undoubted superiority of this method. LVEF significantly improved (increase = 5.0 ± 4.2) in these patients. Also, a significantly improved functional capacity ($p < 0.001$) was confirmed with the 6-minute walk test (6-MWT; 6-months follow-up period; $p < 0.001$) in the CABG plus MNC/SC versus the control (CABG alone) group. This treatment also proved to be a safe therapeutic approach – cardiovascular mortality was significantly lower (5-year follow-up period; $p = 0.049$) in CABG plus MNC/SC patients [2,25,45].

CONCLUSION

SC transplantation is undoubtedly an effective treatment for hematological malignancies and certain immune-mediated disorders. The intensification of the clinical use of cell-mediated therapies (SC transplantation and regenerative medicine) has increased the need for a higher quantity/quality of SCs (better cell yields and viability). Improved SC collection, purification and cryopreservation protocols, as well as improved extracorporeal operating procedures, have minimized mechanical and thermal cell damage during *ex vivo* manipulations. However, it is necessary to have optimized SC extracorporeal manipulations. The implementation of the concept of SC plasticity has stimulated the ever-increasing therapeutic application of immature SCs for organ regeneration/repair. We still need much more fundamental research and a greater number of randomized, controlled and larger clinical trials of SC transplantations, especially in the field of regenerative medicine, in order to investigate the possible role of this revolutionary therapy in treating ischemic heart disease and other tissue/organ damage.

HIGHLIGHTS

The use of conventional SC transplantations is undoubtedly effective in the treatment of hematological patients. The well-known beneficial effects of allogeneic SC transplantations in hemato-oncology have only recently been used to design a system that separates the positive effects of GvL from the negative effects of GvHD.

SC plasticity is a phenomenon of inter-systemic cell plasticity, which represents a wide-ranging phenotypic potential of very primitive SCs capable of homing to different tissues following implantation into a damaged area, with a subsequent transdifferentiation into the cell lineages of the host tissue/organ.

Intersystemic SC plasticity provides a rationale for the use of immature SCs in the treatment of patients with damage to the heart, liver, pancreas, and other organs/tissues.

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