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Matične ćelije zubne pulpe i njihov potencijalni značaj u regenerativnoj medicini

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Dental pulp stem cells - potential significance in regenerative medicine

Vera Todorović[1], Dejan Marković[2], Nadežda Milošević-Jovčić[1], Marijana Petakov[1], Bela Balint[3], Miodrag Čolić[4], Ana Milenković[1,2], Ivana Čolak[1], Vukoman Jokanović, Nebojša Nikolić[2]
[1]Institut za medicinska istraživanja, Univerzitet u Beogradu
[2]Klinika za dečju i preventivnu stomatologiju, Stomatološki fakultet, Univerzitet u Beogradu
[3]Institut za transfuziju, Vojnomedicinska akademija u Beogradu
[4]Institut za medicinska istraživanja, Vojnomedicinska akademija u Beogradu
[5]Institut za nuklearna istraživanja Vinča, Univerzitet u Beogradu

[1] Institute for Medical Research, University of Belgrade
[2]Department of Preventive and Paediatric Dentistry, Faculty of Dentistry, University of Belgrade
[3]Institute for Tranfusion, Military Medical Academy in Belgrade
[4]Institute for Medical Research, Military Medical Academy in Belgrade
[5]Institute of Nuclear Scinces – Vinca, University of Belgrade

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KRATAK SADRŽAJ

Iz zubne pulpe su do danas izolovane tri populacije matičnih ćelija koje su označene kao matične ćelije zubne pulpe (engl. Dental Pulp Stem Cells, DPSC), matične ćelije iz eksfoliranih mlečnih zuba (engl. Stem Cells From Human Exfoliated Decidual Teeth, SHED) i nezrele matične ćelije zubne pulpe (engl. Immature Dental Pulp Stem Cells, IDPC). Sve matične ćelije zubne pulpe su ektomezenhimalnog porekla i lokalizovane su u perivaskularnoj niši. One se lako i efikasno izoluju, visoko su proliferativne, klonogene, multipotentne, ispoljavaju visok stepen plasticiteta i i slične su mezenhimalnim matičnim ćelijama koštane srži (BMSC). U njima je pokazana visoka ekspresija gena alkalne fosfataze, proteina 1 matriksa dentina i dentin-sijalofosfoproteina. Takođe, istaknuta je važnost u ovim ćelijama ekspresije više gena koji kodiraju sintezu komponenti ekstracelularnog matriksa, molekula ćelijske adhezije, faktora rasta, transkripcionih faktora, gena prenosa ćelijskih signala, ćelijske komunikacije i metabolizma.
U uslovima in vitro ili in vivo ove ćelije mogu da se diferenciraju, s određenim međusobnim razlikama, u pravcu odontoblasta, hondrocita, osteoblasta, adipocita, neurona/glije, glatkih i skeletnih mišićnih ćelija, endotelnih ćelija i melanocita. U uslovima in vivo, nakon implantacije, pokazuju različit potencijal za formiranje dentina, ali i koštanog, masnog i nervnog tkiva.

SUMMARY

To date, three types of dental stem cells have been isolated: Dental Pulp Stem Cells (DPSC), Stem Cells From Human Exfoliated Deciduous Teeth (SHED) and Immature Dental Pulp Stem Cells (IDPC). These dental stem cells are considered as mesenchymal stem cells. They reside within the perivascular niche of dental pulp. They are highly proliferative, clonogenic, multipotent and are similar to mesenchymal Bone Marrow Stem Cells (BMSC). Also, they have high plasticity and can be easy isolated. The expressions of the alkaline phosphatase gene, dentin matrix protein 1 and dentinsialophosphoprotein are verified in these cells. Analyses of gene expression patterns indicated several genes which encode extracellular matrix components, cell adhesion molecules, growth factors and transcription regulators, cell signaling, cell communication or cell metabolism.
In both conditions, in vivo and in vitro, these cells have the ability to differentiate into odontoblasts, chondrocytes, osteoblasts, adipocytes, neurons, melanocytes, smooth and skeletal muscles and endothelial cells. In vivo, after implantation, they have shown potential to differentiate into dentin but also into tissues like bone, adipose or neural tissue. In general, DPSCs are considered to have antiinflammatory and immunomodulatory abilities.

Generalno se smatra da DPSC imaju anti-inflamatorno dejstvo i ispoljavaju imunom-odulatorni efekat. Takođe, dovode do imunološke tolerancije ukoliko se implantiraju u alogena tkiva.
Sposobnost inhibicije proliferacije T limfocita ukazuje na njihovo imunosupresivno dejstvo. Matične ćelije zubne pulpe otvorile su nove perspektive u terapijskoj primeni ovih ćelija ne samo u regeneraciji dentina, tkiva periodoncijuma i koštano-zglobnog tkiva kraniofacijalne regije, već i u lečenju neurotraume, autoimunskih oboljenja, infarkta miokarda, mišićne distrofije i oštećenja vezivnog tkiva.

Ključne reči: matične ćelije, zubna pulpa, regeneracija tkiva

After being grafted into allogenic tissues these cells are ableto induce immunological tolerance.
Immunosuppressive effect is shown through the ability to inhibit proliferation of T lymphocytes.
Dental pulp stem cells open new perspectives in therapeutic use not only in dentin regeneration, periodontal tissues and skeletoarticular, tissues of craniofacial region but also in treatment of neurotrauma, autoimmune diseases, myocardial infarction, muscular dystrophy and connective tissue damages.

Keywords: stem cells, dental pulp, tissue regeneration

Uvod

Matične ćelije su ključna podgrupa nespecijalizovanih ćelija organizma u vrlo ranom stadijumu razvića, koje u normalnim uslovima u datom tkivu mogu da se diferenciraju u različite tipove funkcionalno specijalizovanih zrelih ćelija. One igraju važnu ulogu u embrionalnom razviću i organogenezi (embrionalne i fetalne matične ćelije), kao i u tkivnoj homeostazi i regeneraciji (adultne matične ćelije) [1,2].
Matične ćelije se klasifikuju u odnosu na njihovu funkciju (normalne i kancerske matične ćelije) ili izvor izolacije (embrionalne, fetalne, matične ćelije iz krvi pupčanika i adultne matične ćelije). Postojanje humanih adultnih matičnih ćelija do danas je dokazano u koštanoj srži, perifernoj krvi, folikulu dlake, epitelu digestivnog trakta, skeletnom i srčanom mišiću, plućima, retini, mozgu, jetri, pankreasu, masnom tkivu, sinovijumu, periostijumu i zubu[2, 3, 4, 5]. Iako su u relativnom stanju mirovanja, ove ćelije su sposobne da snažno odgovore na tkivno oštećenje.

Najnovija istraživanja pokazuju da embrionalne i neke adultne matične ćelije mogu biti upotrebljene za regeneraciju dentina, tkiva periodoncijuma i rekonstrukciju kraniofacijalnih struktura.
Ne-hematopoetske mezenhimalne matične ćelije koštane srži (engl. Bone Marow Stromal Stem Cells, BMSC/MAPC), koje poseduju multipotentni potencijal i veliki plasticitet, mogu da se diferenciraju u osteoblaste, cementoblaste i ameloblaste, što otvara nove mogućnosti u regenerativnoj stomatologiji[6,7]. Potrebno je istaći pionirski pokušaj i uspeh u kliničkoj upotrebi BMSC i plazme obogaćene trombocitima, upotrebom tehnologije tkivnog inženjeringa, u regeneraciji periodontalnog tkiva ljudi [8]. Takođe, dokazano je da kultivisane matične ćelije masnog tkiva pacova pomešane sa plazmom obogaćenom trombocitima i aplikovane lokalno životinjama sa periodontalnim defektom, indukuju duž alveolarne kosti stvaranje tkiva sličnog periodontalnom ligamentu[9], pa se smatra da će u budućnosti zbog lake dostupnosti masnog tkiva dobijenog liposukcijom, matične ćelije masnog tkiva biti korisne u ćelijskoj terapiji oboljenja periodoncijuma.

Introduction

Stem cells are unspecialized cells in early stage of development which have, in normal condititions, the ability to differentiate into a diverse range of specialized mature cells. They play important role in embryonic development and organogenesis (embryonic and fetal stem cells) as well as in homeostasis and tissue regeneration (adult stem cells)[1,2]
Stem cells can be classified due to their function (normal and cancer stem cells) or potential source of isolation (embryonic, fetal, umbilical cord, adult stem cells). To date, adult stem cells have been isolated from many tissues including bone marrow, periferal blood, hair follicle, digestive tract epithelium, skeletal and cord muscle, lungs, retina, brain, liver, pancreas, adipose tissue, synovium, periost and tooth[2, 3, 4, 5]. Although, these cells are in relatively inactive stage, they are capable of responding to tissue damage.

Recent advances in stem cell biology and gene therapy technology have provided the great potential of embryonic and some adult stem cells for use in dentin regeneration, periodontal tissue regeneration and reconstruction of cranifacial structures.
Non-hematopoietic mesenchymal BMSC posses multilineage potential and plasticity so they are able to differentiate into osteoblasts, cementoblasts and ameloblasts[6, 7]. These findings promise significant implications for regenerative dentistry. Also, recent study has demonstrated successful periodontal tissue regeneration with mesenchymal stem cells and platelet-rich plasma using tissue engineering technology[8]. Adipose Stem Cells (ASCs) were isolated from a rat, mixed with platelet-rich plasma obtained from inbred rats, and implanted into the periodontal tissue defect that had been generated in the test rats. Few weeks after implantation, a periodontal ligament-like structure was observed along with alveolar bone[9]. These observations suggest that ASCs can promote periodontal tissue regeneration in vivo. Because large amounts of human lipoaspirates are readily available, ASCs may be useful in future clinical cell-based therapy for periodontal diseases.

Smatra se da će embrionalne matične ćelije u budućnosti biti jedan od alternativnih izvora za rekonstrukciju kraniofacijalnih struktura, s obzirom da je dokazan njihov osteogeni potencijal u uslovima kultivisanja, dok se u uslovima in vivo ove ćelije diferenciraju u koštano tkivo u zubnoj alveoli pacova, bez pojave teratoma[10].

Matične ćelije zubne pulpe lokalizovane su u perivaskularnoj niši i slične su BMSC. One su visoko proliferativne, klonogene, multipotentne i ispoljavaju visok stepen plasticiteta. Zbog lake dostupnosti, smatra se da će zubna pulpa, pored koštane srži, u budućnosti predstavljati najvažniji izvor adultnih multipotentnih mezenhimalnih matičnih ćelija, koje mogu da nađu široku primenu u regenerativnoj medicini[11, 12].

Izolacija, klasifikacija, molekulske i funkcionalne karakteristike matičnih ćelija zubne pulpe

Matične ćelije zuba mogu se izolovati iz: 1) zubnog epitela, 2) zubne pulpe, 3) periodontalnog ligamenta, 4) zubnog folikula i 5) apikalne papile zuba. Sve matične ćelije zuba, izuzev zubnih epitelnih matičnih ćelija koje se gube kod ljudi nakon izbijanja zuba i potiču od ektoderma, su ektomezenhimalnog porekla i potiču iz nervnog grebena. Osnovni potencijal diferencijacije matičnih ćelija zuba leži u njihovoj sposobnosti da formiraju dentin i tkiva periodoncijuma[13].

Veoma je kompleksna karakterizacija različitih matičnih ćelija zuba na osnovu eksperesije površinskih proteinskih markera, kao i genske ekspresije (šema 1, tabela 1)[13, 14].

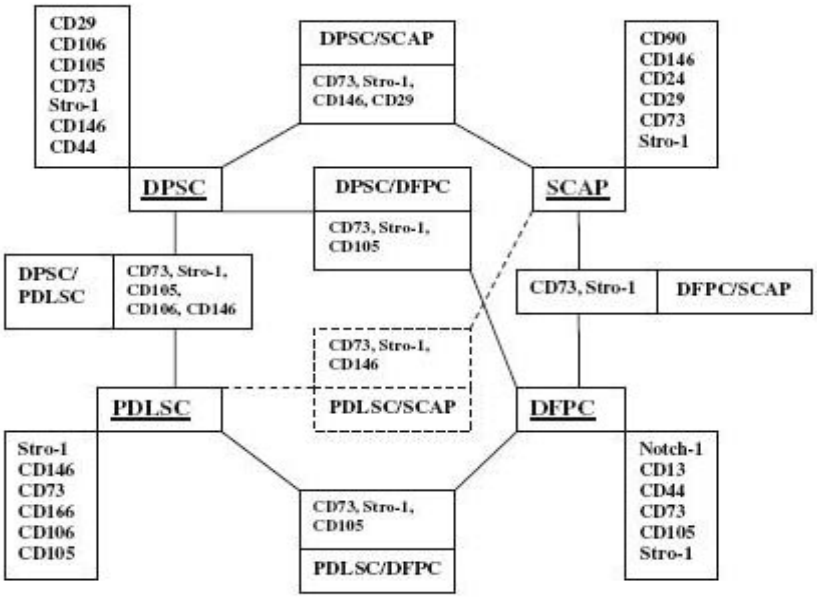
In recent study, the osteogenic potential of Embryonic Stem Cells (ESCs), using in vitro culture conditions was assessed. In vivo differentiation showed formation of osteogenic structure in the tooth sockets without evidence of teratomas[10]. These data suggest that pluripotent ESCs can serve as an alternative source for the reconstruction of craniofacial structures.

Dental pulp stem cells reside within the perivascular niche of dental pulp and are considered as mesenchymal stem cells. They are highly proliferative, clonogenic, multipotent and also have high plasticity. Extraction of stem cells from pulp tissue is highly efficiency. The easy management of dental pulp stem cells make them, beside bone marrow stem cells, feasible for use in clinical trials on human patients and regenerative medicine[11, 12].

Isolation, classification, molecular and functional characteristics of dental pulp stem cells

Dental tissues which have been identified as accessible sources of stem cells are: 1) dental epithelium, 2) dental papilla, 3) periodontal ligament, and 4) dental follicle. All dental stem cells are neural crest-derived ectomesenchymal cells, with the exception of those from dental epithelium which are ectodermal cells. Constructing complex structures like dentin and periodontal tissue, dental stem cell biology might provide meaningful insights into the development of dental tissues and cellular differentiation processes [13].

Characterization of diverse types of dental stem cells, due to expression of surface protein markers as well as gene expression, is very complexed (figure 1, table 1)[13, 14].



DPSC-Dental Pulp Stem Cell; DFPC-Dental Follicular Precursor Cell; SCAP-Stem Cell of Apical Papilla; PDLSC-Periodontal Ligament Stem Cell

Šema 1. Najvažniji površinski ćelijski markeri matičnih ćelija zuba*
* po Morsczeck et al., Clin Oral Invest 2008; 12:113-118

Antigen	DPSC*	SHED	PDLSC	BMSC
CD14	-	-	-	-
CD34	-	-	-	-
CD44	++	++	++	++
CD45	-	-	-	-
CD106	+	+/-	+/-	++
CD146	++/+/-	++/+/-	++/+/-	++/+/-
3G5	+/-	+/-	+/-	+/-
Stro-1	++/+/-	++/+/-	++/+/-	++/+/-
α-glatkomišićni aktin	++/-	++/-	++/-	++/+/-
kolagen tip-I	++	++	++	++
kolagen tip-III	++/+	++/+/-	++/+/-	++/+
alkalna fosfataza	++/+/-	++/+/-	++/+/	++/+/-
osteokalcin	++/+	++/+/-	++/-	+/-
osteonektin	++/+	++/+	++/+	++/+
osteopontin	+/-	+/-	+/-	+/-
sialoprotein kosti	-	-	-	-
skleraksis	+	+	++	+
sialofosfoprotein dentina	-	-	-	-

*DPSC-Dental Pulp Stem Cells; SHED-Stem Cells From Human Exfoliated Decidual Teeth; PDLSC-Periodontal Ligament Stem Cells; (++) jaka ekspresija; (+) slaba ekspresija; (-) negativno; (/) subpopulacija

Tabela 1. Profil ekspresije proteina ili genske ekspresije u nekim matičnim ćelijama zuba u uslovima kultivisanja in vitro i odnos prema BMSC

Međutim, neki površinski proteinski markeri, kao što su Stro-1 i CD73 su ubikvitarno eksprimirani na svim matičnim i prekursorskim ćelijama zuba[13]. Posebna populacija matičnih ćelija zubne pulpe označena kao nezrele matične ćelije zubne pulpe (engl. Immature Dental Pulp Stem Cells, IDPC) eksprimira markere embrionalnih matičnih ćelija Oct-4, Nanog, SSEA-3, SSEA-4, TRA-1-60 i TRA-1-81. Pretpostavlja se da su ove ćelije u stvari pluripotentni prekursori druge dve populacije matičnih ćelija zubne pulpe označenih kao matične ćelije zubne pulpe u užem smislu reči (engl. Dental Pulp Stem Cells, DPSC) i matičnih ćelija zubne pulpe izolovanih iz eksfoliranih mlečnih zuba (engl. Stem Cells From Human Exfoliated Decidual Teeth, SHED)[15].

Regulacija homeostaze adultnih matičnih ćelija rezultat je suptilnog balansa između genetičkih i molekularnih ćelijskih mehanizama, spoljašnjih faktora iz lokalnih i sistemskih niša tela, kao i različitih puteva prenosa ćelijskih signala. Određivanjem profila genske ekspresije DPSC i funkcionalnom klasifikacijom tih gena ustanovljena je, pre svega, visoka ekspresija gena alkalne fosfataze, proteina 1 matriksa dentina (DMP1) i dentin-sijalofosfoproteina. Takođe, ukazano je na važnost u ovim ćelijama ekspresije više gena koji kodiraju sintezu komponenti ekstracelularnog matriksa, molekula ćelijske adhezije, faktora rasta i transkripcionih faktora. Funkcionalna analiza i analiza klasteriranja ukazala je važnost ekspresije gena prenosa ćelijskih signala, ćelijske komunikacije i ćelijskog metabolizma (tabela 2)[14].

Some surface proteins, like Stro-1 and CD73, are ubiquitously expressed by all dental stem or precursor cells[13]. Population of Immature Dental Pulp Stem Cells (IDPSC) express embryonic stem cell markers Oct-4, Nanog, SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81. It is assumed that these cells are pluripotent precursors to the other two stem cell populations known as Dental Pulp Stem Cells (DPSC) and Stem Cells From Human Exfoliated Deciduous Teeth (SHED)[15].

To maintain homeostasis, a balance between genetic and molecular cell mechanism, extrinsic signals from the microenvironment (called niche) in which stem cells reside and signaling pathway in regulation of stem cell properties, is essential. Determining the profile of gene expression of dental pulp stem cells and functional classification of these genes, the expressions of the alkaline phosphatase gene, dentin matrix protein 1, and dentinsialophosphoprotein are verified and consequently the high expressions of these genes are discovered. Analyses of gene expression patterns indicated several genes which encode extracellular matrix components, cell adhesion molecules, growth factors, and transcription regulators. Functional and clustering analyses of differences in gene expression levels revealed cell signaling, cell communication, or cell metabolism (table 2)[14].

Glavna kategorija gena	Podkategorija gena	hBMSC (I)*		hDPSC		hDPSC (I)		hOMC		hDPSC (I) (18–24)	
		↑	↓	↑	↓	↑	↓	↑	↓	↑	↓
deoba ćelije	DNK sinteza/replikacija	8	7	4	9	7	6	12	2	2	
	apoptoza	15	6	39	20	31	16	39	12	11	6
	ćelijski ciklus	25	8	27	31	39	15	68	7	11	14
	hromozom	6	5	4	9	5	3	9	1	1	3
	ikupno	54	26	74	69	82	40	128	22	25	23
signalni putevi ćelije ili ćelijska komunikacija	ćelijska athezija	16	14	33	22	23	24	32	26	10	4
	kanali/transport proteina	6	9	13	11	18	14	18	8	7	6
	faktori rasta/citokini	15	9	13	8	23	6	30	7	16	3
	metabolizam	18	12	17	20	28	14	42	8	10	4
	modifkacija proteina	5	8	20	18	23	3	12	10	14	9
	receptori	59	32	78	53	65	44	81	62	28	16
	Ukupno	119	84	174	132	180	105	215	121	85	42
struktura ćelije / motilitet	citoskelet	4	3	5	7	6	5	11	2	2	5
	ekstracelularni matriks	30	11	17	25	15	31	15	31	5	7
	protein vezan za mikrotubule	1		7		5		14			3
	ukupno	35	14	29	32	26	36	40	33	7	15
ćelija / odbrana organizma	homeostaza (odgovor na stres)	41	14	40	45	61	25	56	39	28	16
	imunologija (imuni odgovor)	46	10	41	30	53	17	51	46	24	11
	ukupno	87	24	81	75	114	42	107	85	52	27
gen / ekspresija proteina	RNK sinteza (transkripcioni faktor)	31	16	62	49	52	21	55	31	35	13
	sinteza proteina (vezivanje proteina)	4	3	3	7	6	2	5	2	2	5
	ukupno	35	19	65	56	58	23	60	33	37	18
metabolizam	aminokiselina	5	1	7	4	9	5	3	5	7	1
	energija/TCA ciklus	1	1	3		3		4			
	lipid	21	4	27	7	33	6	21	6	13	1
	nukleotid	2	2	4	7	6	4	21	8	4	8
	modifkacija proteina	13	12	41	32	20	3	13	21	19	11
	transport	45	19	50	39	83	32	62	35	39	12
	ukupno	87	39	132	89	154	50	124	75	82	33
	UKUPNO	417	206	555	453	614	296	674	369	288	158

*hBMSC (I)-humane mezenhimalne matične ćelije koštane srži u osteoidnuktivnoj sredini 18. dana kultivacije; hDPSC (I)-humane matične ćelije zubne pulpe u osteoinsuktivnoj sredini 18. dana kultivacije; hOMC-humane ćelije oralne mukoze; hDPSC (I) (18-24)- humane matične ćelije zubne pulpe u osteoinsuktivnoj sredini 18-24 dana kultivacije

Tabela 2. Funkcionalna klasifikacija gena hDPSC

Što se tiče razlika u genskoj ekspresiji između MAPC/BMSC i DPSC, one se ogledaju pre svega u povećanoj ekspresiji kod DPSC gena koji kontrolišu ćelijski ciklus, naročito gena ciklin-zavisne kinaze-6 koja je aktivator ćelijskog ciklusa, što objašnjava višu stopu proliferacije DPSC u odnosu na MAPC/BMSC[14, 16]. Put prenosa signala Wnt/beta-katenin[17], koji je ključan za samoobnavljanje matičnih ćelija, kao i put Noch[18], negativno regulišu diferencijaciju in vitro DPSC u odontoblaste, dok induktivni efekat imaju FGF2 i TGFbeta1[19]. U poslednje vreme poznato je da je hipoksija jedan od ključnih faktora u održavanju nediferenciranog stanja i plasticiteta adultnih matičnih ćelija[20]. Međutim, uslovi kultivisanja koji imitiraju ishemiju (hipoksiju i hipoglikemiju) nepovoljno utiču na preživljavanje i diferencijaciju DPSC[21]. Bolje poznavanje ekspresije površinskih markera i gena matičnih ćelija zubne pulpe, puteva prenosa signala, i njihov odnos prema ćelijskoj diferencijaciji i plasticitetu, svakako će u budućnosti doprineti razvoju terapije bazirane na primeni ovih ćelija u humanoj medicini i stomatologiji.

Matične ćelije zubne pulpe imaju sve osobine potrebne za uspešnu terapijsku primenu: 1) lako su dostupne za izolaciju; 2) izolacija je veoma efikasna; 3) imaju multipotentni potencijal; 4) pokazuju interakciju sa biomaterijalima koji upotrebljeni kao matrice indukuju intenzivnu proliferaciju ovih ćelija; 5) dugovečne su i 6) podležu uspešnoj krioprotekciji, slično drugim matičnim ćelijama[11, 22]. Podatak da vijabilne matične ćelije zubne pulpe mogu da se izoluju i nakon 5 dana od ekstrakcije zuba, kao i da ih je moguće izolovati i kultivisati iz intaktnih zuba koji su prethodno krioprezervirani, ukazuje da su potrebni minimalni zahtevi za stvaranje banke ovih ćelija u cilju buduće terapijske primene[12].

Studijama in vitro je dokazano da matične ćelije zubne pulpe imaju izrazit plasticitet, tj. sposobnost da se diferenciraju u ćelije različite od ćelija prisutnih na mestu odakle potiču, a najnoviji radovi pružaju dokaze o njihovom plasticitetu i u uslovima in vivo. U uslovima in vitro ili in vivo ove ćelije mogu da se diferenciraju, s određenim međusobnim razlikama, u pravcu odontoblasta, hondrocita, osteoblasta, adipocita, neurona/glije, glatkih i skeletnih mišićnih ćelija, endotelnih ćelija i melanocita[15, 16, 23-29]. U uslovima in vivo, nakon implantacije, pokazuju različit potencijal za formiranje dentina, ali i koštanog i masnog tkiva[30].

Generalno se smatra da DPSC, slično drugim mezenhimalnim matičnim ćelijama, imaju anti-inflamatorno dejstvo. One ispoljavaju imunomodulatorni efekat jer mogu biti uključene u imunski odgovor u toku infekcije zubne pulpe, a verovatno i periodoncijuma, koji je veoma kompleksan[31, 32], putem aktivacije NF-□B[33]. Takođe, dovode do imunološke tolerancije ukoliko se implantiraju u alogena tkiva[11].

Comparing the gene expression profiles of human dental pulp stem cells to bone marrow stromal stem cells a few differentially expressed genes, including cell cyclus activator cyclin-dependent kinase 6, were highly expressed in DPSCs. This explains the higher proliferation rate of dental pulp stem cells than bone marrow stromal stem cells[14, 16]. Wnt/beta-katenin, important for stem cells self-renewal[17], and Notch signaling[18] can inhibit the odontoblastic differentiation, while two crucial growth factors, FGF2 and TGFbeta1[19], have inductive effects on the odontoblastic differentiation of human dental pulp stem cells in vitro. Although differentiated and undifferentiated cells can be exposed to ischemic conditions in cases of injury or inflammation, the effects of ischemia on cell survival and differentiation have not been well characterized[20]. These data showed that the ischemic conditions have similar detrimental influence on both undifferentiated and differentiated porcine Dental Pulp-derived Cells (pDPCs), and affect differentiation status of pDPCs[21].

Dental pulp stem cells have all characteristics for successful therapeutic use: 1) access to the collection site of these cells is easy, 2) extraction of stem cells from pulp tissue is highly efficiency; 3) they have an extensive differentiation ability, 4) demonstrated interactivity with biomaterials makes them ideal for tissue reconstruction, 5) they have a long lifespan, 6) they can be safely cryopreserved[11, 22]. Recent studies indicate that DPSC isolation is feasible for at least 5 days after tooth extraction. Further, the recovery of viable DPSC after cryopreservation of intact teeth suggests that minimal processing may be needed for the banking of samples with no immediate plans for expansion and use[12].

An increasing number of investigations supports that stem cells have the potential to differentiate into matured cell types beyond their origin, a property defined as plasticity. Previously, the plasticity of DPSCs has been confirmed by culturing cells in lineage-specific media in vitro. In recent studies, it has been confirmed in vivo conditions as well. In both conditions, in vivo and in vitro, these cells have the ability to differentiate into odontoblasts, chondrocytes, osteoblasts, adipocytes, neurons, melanocytes, smooth and skeletal muscles and endothelial cells[15, 16, 23-29]. In vivo, after implantation, they have shown potential to differentiate into dentin but also into bone or adipose tissue[30].

In general, DPSCs are considered to have anti-inflammatory and immunomodulatory abilities because they may be involved in immune responses during pulpal infection, and probably periodontium as well[31,32], through activating NF-□B 33. After being grafted into allogenic tissues these cells are able to induce immunological tolerance[11].

Sposobnost inhibicije proliferacije T limfocita ukazuje na njihovo imunosupresivno dejstvo i moguću primenu kod autoimunskih bolesti[34].

Različite vrste matičnih ćelija zubne pulpe i njihov potencijalni značaj u regenerativnoj medicini i stomatologiji

Sredinom devedesetih godina XX. veka došlo je do uspešne izolacije prekursorskih ćelija zubne pulpe[35]. Kasnije su matične ćelije izolovane iz zubne pulpe kutnjaka i nazvane DPSC[23], kao i iz eksfoliranih mlečnih zuba–SHED[36] i IDPC[15].

DPSC

Ove ćelije su do sada izdvojene iz umnjaka, mlečnih sekutića i prekobrojnih zuba[16, 23, 37]. Kao što je već iustaknuto, ispoljavaju slične karakteristike kao BMSC[38].

Prva istraživanja na DPSC izolovanim iz umnjaka su pokazala da se ove ćelije diferenciraju u odontoblaste, adipocite i u neuronima-slične ćelije i da ta diferencijacija podseća na embrionalnu ontologiju kranijalnih ćelija nervnog grebena. Istovremeno je pokazano i da imaju neke karakteristike identične sa osteoblastima[24]. Međutim, najnovija istraživanja pokazuju da humane (h) DPSC imaju daleko veći potencijal diferencijacije, s obzirom da mogu da se diferenciraju u ćelije mezenhimalnog porekla (odontoblaste, osteoblaste, adipocite, hondrocite i poprečnoprugaste mišićne ćelije) ali i u melanocite (ćelije koje nisu porekla mezenhima, već potiču od ćelija nervnog grebena)[26]. Stoga se pretpostavlja da se u kulturama hDPSC nalaze i ćelije koje bi mogle biti multipotentne matične ćelije nervnog grebena.

Pokazano je da humane DPSC izolovane iz mlečnih zuba, koje u uslovima in vitro pokazuju osteogeni, adipogeni i miogeni potencijal diferencijacije, transplantirane pacovima sa velikim defektom parijetalne regije, dovode do stvaranja nove kosti nakon 1 meseca od načinjene lezije, bez upotrebe imunosupresivne terapije[25]. To daje nadu da će se ove ćelije u budućnosti moći upotrebiti u rekonstruktivnoj hirurgiji kraniofacijalne regije.

Matične ćelije označene kao SBP-DPSC su multipotentna subpopulacija DPSC, koja je sposobna da se diferencira u osteoblaste i da sintetiše trodimenzionalnu lamelarnu kost in vitro. Ukoliko se SBP-DPSC ili koštani model dobijen iz njih u uslovimsa in vitro transplantira pacovu sa deficitom imunološkog sistema stvoriće se tkivna struktura sa integralnim krvnim sudovima, slična pravoj kosti odraslih ljudi[28,29]. Dokazano je da čak i nakon 2 godine od krioprezervacije SBP-DPSC zadržavaju sposobnost diferencijacije, proliferacije i sinteze koštanog tkiva[29].

Immunosuppressive effect and possible therapeutic use in autoimmune diseases is shown through the ability to inhibit proliferation of T lymphocytes[34].

Different types of dental pulp stem cells - potential significance in regenerative medicine and dentistry

In the last decade of the twentieth century, precursor cells of dental pulp were successfully isolated[35]. Furthermore, DPSCs[23] were isolated from dental pulp of human adult third molar as well as from human exfoliated deciduous teeth[36] and immature dental pulp [15].

DPSC

To date, these cells have been isolated from third molars, decidual incisors and supernumerary teeth[16, 23, 37]. DPSC have similar characteristics to Bone Marrow Stem Cells (BMSC)[38].

DPSCs derived from third molars were also found to be capable of differentiating into odontoblasts, adipocytes and neural-like cells and that differentiation reminiscent of cranial neural crest (CNC) cells embryonic ontology. At the same time, it is found that DPSCs have some characteristics identical to osteoblasts[24]. It is demonstrated that human dental pulp contains self renewing human Dental Pulp Stem Cells (hDPSCs) capable of differentiating into mesenchymal-derived odontoblasts, osteoblasts, adipocytes, chondrocytes and striated muscle, and interestingly, also into non-mesenchymal, neural crest- derived melanocytes 26. Furthermore, this study showed that hDPSC cultures include cells with traits attributed to multipotent stem cells, and provide evidence that these might be multipotent neural crest stem cells.

Human dental pulp stem cells, were isolated from deciduous teeth, to reconstruct large-sized cranial bone defects in nonimmunosuppressed rats. They showed osteogenic, adipogenic, and myogenic in vitro differentiation[25]. These findings suggest that hDPSC is an additional cell resource for correcting large cranial defects in rats and constitutes a promising model for reconstruction of human large cranial defects in craniofacial surgery.

Stromal stem cells from human dental pulp (SBP-DPSCs) are multipotent stem cells able to differentiate into osteoblasts, which synthesize three-dimensional woven bone tissue chips in vitro. When either SBP-DPSCs or bone chips obtained in vitro were transplanted into immunocompromised rats, they generated a tissue structure with an integral blood supply similar to that of human adult bone[28,29]. After storage for 2 years, it is found that stem cells are still capable of differentiation, and that their differentiated cytotypes proliferate and produce woven bone tissue[29].

Subpopulaciju DPSC, označenu kao SP-DPSC imaju veliki angiogenetski potencijal. Ukoliko se miševima sa ishemijom prednje šape lokalno transplantiraju ove ćelije doći će do njihovog uspešnog kalemljenja, povećanja gustine kapilarne mreže i bolje prokrvljenosti ishemijske zone, a kondicionirani medijum od ovih ćelija pokazuje mitogenu i anti-apoptotsku aktivnost na endotelne ćelije humane umbilikalne vene[39].

Stvaranje in vivo tkiva sličnog zubnoj pulpi, nakon subkutane aplikacije mišu trijade DPSC, matrice od kolagena i DMP1, ukazuje na veliki potencijalni značaj ovih ćelija u tkivnom inženjeringu [40].

SHED

Matične ćelije iz eksfoliranih mlečnih zuba su sposobne za diferencijaciju u odontoblaste, adipocite i nervne ćelije. U uslovima in vivo one indukuju formiranje kosti i produkciju dentina; nakon transplantacije u miša sa kompromitovanim imunološkim sistemom migriraju u mozak i sposobne su za preživljavanje 36. Studije in vivo su takođe pokazale da se humane SHED diferenciraju u odontoblaste i endotelne ćelije i da stoga eksfolirani mlečni zubi predstavljaju vijabilni izvor matičnih ćelija za tkivni inženjering zubne pulpe. Naime, ukoliko se hSHED oblože biodegradibilnim materijalom pripremljenim sa presecima normalnog zuba i kao takve implantiraju mišu sa imunološkim deficitom, oformiće se tkivo koje u histološkom pogledu i u pogledu ćelijskog sastava veoma podseća na zubnu pulpu [41].

IDPC

Radi se o najprimitivnijoj populaciji matičnih ćelija zubne pulpe, s obzirom da je pokazano da eksprimiraju markere embrionalnih matičnih ćelija. U toku kultivisanja kroz 25 pasaža, u toku 4 meseca, one zadržavaju normalni kariotip i stopu ekspanzije karakterističnu za matične ćelije. U hemijski definisanim uslovima kultivisanja in vitro, IDPC se diferenciraju u glatke i poprečnoprugaste mišićne ćelije, neurone, hondrocite i osteoblaste. U uslovima in vivo, ukoliko se transplantiraju miševima sa imunološkim deficitom, dobro se kaleme u različitim tkivima 15. Aplikacija hIDPC bez primene imunosupresivne terapije, pokazala se uspešnom u kontroli mišićne distrofije kod zlatnih retrivera (najboljeg animalnog model za Duchenne-ovu mišićnu distrofijiju kod ljudi), pri čemu je sistemska aplikacija ovih matičnih ćelija bila bolji izbor od lokalne aplikacije [42].

Zaključak

Matične ćelije zubne pulpe otvorile su nove perspektive u terapijskoj primeni ovih ćelija ne samo u regeneraciji dentina, tkiva periodoncijuma i koštano-zglobnog tkiva kraniofacijalne regije, već i u lečenju neurotraume, infarkta miokarda, mišićne distrofije i oštećenja vezivnog tkiva.

Side population (SP) dental pulp stem cells, SP-DPSC, has high angiogenetic potential. In models of mouse hindlimb ischemia, local transplantation of one subfraction of SP cells resulted in successful engraftment and an increase in the blood flow including high density of capillary formation. Conditioned medium from this subfraction showed the mitogenic and anti-apoptotic activity on human umbilical vein endothelial cells [39].

In vivo generation of dental pulp-like tissue by using the triad of dental pulp stem cells, a collagen scaffold, and dentin matrix protein 1 after subcutaneous transplantation in mice, might lead to hard tissue formation. This finding indicate that these cells might have high potential significance in tissue engineering [40].

SHED

SHED were identified to be a population capable of differentiating into a variety of cell types including neural cells, adipocytes, and odontoblasts. After in vivo transplantation, SHED were found to be able to induce bone formation, generate dentin and survive in mouse brain along with expression of neural markers 36. SHED differentiated into odontoblast-like cells in vivo and in endothelial-like cells. SHED seeded in biodegradable scaffolds prepared within human tooth slices and transplanted into immunodeficient mice result in tissue with architecture and cellularity that closely resemble those of a physiologic dental pulp. This work suggests that exfoliated deciduous teeth constitute a viable source of stem cells for dental pulp tissue engineering [41].

IDPC

The population of immature dental pulp stem cells is dental pulp stem cell population which expresses embryonic stem cell markers during at least 25 passages while maintaining the normal karyotype and the rate of expansion characteristic of stem cells. Moreover, in vitro these cells can be induced to undergo uniform differentiation into smooth and skeletal muscles, neurons, cartilage, and bone under chemically defined culture conditions. After in vivo transplantation of these cells into immunocompromised mice, they showed dense engraftment in various tissues 15. Human Immature Dental Pulp Stem Cells (hIDPSC) were transplanted, without any immunosuppression, into golden retriever muscular dystrophy dogs, who represent the best available animal model for therapeutic trials aiming at the future treatment of human Duchenne muscular dystrophy. Data from this trial suggested that systemic multiple deliveries seemed more effective than local injections. These findings open important avenues for further researches [42].

Conclusion

Dental pulp stem cells open new perspectives in therapeutic use not only in dentin regeneration, periodontal tissues and skeletoarticular tissues of craniofacial region but also in treatment of neurotrauma, autoimmune diseases, myocardial infarction, muscular dystrophy and connective tissue damages.

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Adresa za korespondenciju
dr sci. med. Vera Todorović, naučni savetnik
Institut za medicinska istraživanja,
Univerzitet u Beogradu
Dr Subotića 4, 11129 Beograd, PF 129
e-mail: verat@imi.bg.ac.yu
Tel:011/2685-788/L-111 ili 113

Address for correspondence
Vera Todorovic, research professor
Institute for medical research
University of Belgrade
Dr Subotica 4 11000 Belgrade, PO BOX 129
e-mail: verat@imi.bg.ac.yu
Tel:+381 11/2685-788/ L-111 ili 113