

FIBROKLAST KAO POMAŽUĆI FAKTOR KOLAGENOLIZE KOD PARODONTOPATIJA DIJABETIČARA

FIBROCLAST AS A HELPING FACTOR OF COLLAGENOLYSIS IN PERIODONTAL DISEASE OF DIABETIC PATIENTS

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Kratak sadržaj

Iako najčešće započinje kao zapaljenjski proces bakterijske geneze, inflamacija gingive kod parodontopatije vremenom postaje autoagresivna bolest periodonta. Kod dijabetičnih bolesnika ubrzana je progresija ovog oboljenja zbog pada opšteg i lokalnog imuniteta. Cilj ovog rada je da se elektronomikroskopski pokaže prisustvo ćelije čije se ultrastrukturne karakteristike izdvajaju iz grupe postojećih ćelijskih tipova fibroblastnog ćelijskog niza ili su ćelijska modulacija fibroblasta. Materijal i metode: Ispitivanje je izvršeno na jednom slučaju bolesnika muškog pola, starosti 50 godina, koji je bolovao od parodontopatije zadnjih 15 godina, i dijabetes tip II, u vremenskom trajanju od 10 godina. Posle ekstrakcije prvog donjeg molara sa desne strane, uzet je biopsijski uzorak gingive, koji je fiksiran u osmijum tetratoksidu i obrađen odgovarajućom standardnom procedurom, a potom posmatrani pod elektronskim mikroskopom JEOL 100.

Rezultati: Elektronomikroskopski zapažene su varijante fibroblasta, kao i ćelije koje po spoljašnjoj formi nalikuju fibroblastima i fibroцитima, ali se jedna podvarijanta ovih ćelija ultrastrukturno razlikuje od drugih ćelija fibroblastnog niza po svom organelnom sadržaju. Te ćelije su duguljaste, sa po nekim citoplazmatskim produžetkom. Citoplazma pokazuje neuobičajeno prisustvo i raspored organela. Sa druge strane, primarni i sekundarni lisozomi prisutni su u neobičnoj količini za fibroblaste i fibrocite. Postoji i mogućnost da je fibroblast indukovana ćelija od stem ćelije fibroblasta, a ne transformisani već postojeći diferentovani fibrocit.

Zaključak: Visokoagresivna kolagenoliza gingive i periodoncijuma kod parodontopatije dijabetičara, indukuje razvoj nove ćelije, fibrolasta, koja istovremeno raspolaže sposobnošću kolagenosinteze lučenjem proteolitičkih enzima u ekstracelularni matriks i fagocitizom i intracellularnom degradacijom kolagenih vlakana.

Ključne reči: fibroblast, parodontopatija, dijabetes

Abstract

Although it most frequently begins as an inflammatory process of bacterial genesis, gingivitis with periodontal disease in time becomes an autoaggressive illness of the periodontium. Progression of this illness is sped up with diabetic patients due to the fall of general and local immunity.

The aim of work is to show electron microscopically the presence of a cell which ultrastructural characteristics distinguish themselves from the group of existing cell types of fibroblast cell series or are a cell modulation of the fibroblast.

Material and methods: The examination has been carried out on one patient who has been suffering periodontal disease for 15 years and type II diabetes for 10 years. Biopsy samples of the gingiva were examined using EM JEOL 100.

Results: By EM variants of fibroblast are noticed as well as the cells alike fibroblasts and fibrocytes by their outer form, but one subvariant of these cells ultrastructurally differs from the other cells of fibroblast series in its organel contents.

Namely, those cells are longish, with some cytoplasm extinctions. The cytoplasm shows unusualness of presence and arrangement of organelles. On the other hand, primary and secondary lysosomes are usually present, in an unusual quantity for fibroblasts and fibrocytes. There is also a possibility that a fibroblast is an induced cell from the stem cell of fibroblast, and not transformed but existing differentiated fibrocyte.

Conclusion: Highly aggressive collagenolysis of the gingiva and periodontium in periodontal diseases in diabetics induces the development of a new cell, fibroblast, which, at the same time, has the capacity of collagen synthesis.

Key words: fibroblast, periodontal disease, diabetes mellitus

Uvod

Iako najčešće započinje kao zapaljenjski proces bakterijske geneze, inflamacija gingive kod parodontopatije vremenom postaje autoagresivna bolest periodonta, koja se karakteriše ekscesivnom kolagenolizom postojećih tkiva, koja u svom sastavu imaju 30–40% kolagena od ukupne suve težine organa.¹ Kod dijabetičnih bolesnika ubrzana je progresija ovog oboljenja zbog pada opšteg i lokalnog imuniteta, kompromitovanja cirkulacije endoproliferativnom obliteracijom kapilara, većom sklonosću ka infekciji, dejstvom štetnih metaboličkih produkata stvorenih u toku dijabeta i dr.¹⁻³

Normalno, produkcija kolagena uravnotežena je sa kolagenolizom u čemu učestvuju proteaze, kolagenaze, hijaluronidaza i dr. do spele iz fibroblasta i, eventualno makrofaga. Kod zapaljenjskih procesa kolagenaze se oslobađaju iz polimorfonuklearnih leukocita, makrofaga, fibrocita i drugih ćelija. Kod dijabetičara koji boluju od parodontopatije povećana je kolagenolitična aktivnost. Smatra se da kod dijabeta na sintezu kolagena utiče i hipoglikemija, koja smanjuje proliferaciju i rast fibroblasta, sintezu kolagena i glikozaminoglikana³⁻⁵, što je eksperimentalno potvrđeno na gingivalnim i periodontalnim fibroblastima nekih životinja. Takođe je od značaja i izostanak uticaja smanjene količine inzulina na receptore fibroblasta.^{1,2,6-8} Kod zapaljenjskih procesa dolazi do oslobađanja citokina i faktora rasta, koji, sa svoje strane, podstiču proliferaciju i funkcionalnu angažovanost mnogih ćelija koje su potrebne za kolagenosintezu i kolagenolizu. U procesu kolagenolize, Sehter i Milanova opisuju ćeliju veoma sličnu fibroblastu koja je potekla od istog ćelijskog tipa, ali, koja se karakteriše dvostrukom ulogom i specifičnom funkcijom.⁶ Naime, u citoplazmi ćelije nalazi se obilje cisterni granularnog endoplazmatičnog retikuluma (RER), aparata za kolagenosintezu, a sa druge strane, prisustvo velikog broja primarnih i sekundarnih lizozoma sa kolagenim vlaknima u njima, u različitim stadijumima lize ovih struktura.⁶

Cilj rada

Cilj ovog rada je da se elektronomikroskopski pokaže prisustvo ćelije čije se ultrastrukturne

Introduction

Gingivitis with periodontal disease, although it most frequently begins as an inflammatory process of bacterial genesis, eventually becomes an autoaggressive disease of the periodontium, characterized by an excessive collagenolysis of the existing tissue, which containing 30–40% of collagen out of total dry weight of the organ¹. Progression of this disease is accelerated in diabetic patients due to the fall of general and local immunity, affected circulation by an endoproliferative obliteration of capillaries, greater susceptibility to infections, and influence of harmful metabolic products of disintegration made during the course of diabetes etc.¹⁻³

Normally, the production of collagen is balanced with collagenolysis, including the participation of proteases, collagenase, hyaluronidase etc. produced by fibroblasts and, possibly, macrophages. In the inflammatory processes, collagenases are released from polymorphonuclear leukocytes, macrophages, and other cells. Collagenolytic activity is increased in periodontal disease of diabetic patients. It is considered that in diabetes the synthesis of collagen is influenced by hypoglycemia, which reduces proliferation and the growth of fibroblast, synthesis of collagen and glycosaminoglycan^{3,4,5}, and that has been experimentally confirmed on gingival and periodontal fibroblasts of some animals. Influence of reduced quantity of insulin on the fibroblast receptors also have important significance.^{1,2,6-8}

In the inflammatory processes, released cytokines and the growth factors, urge proliferation and functional engagement of many cells needed for collagen synthesis and collagenolysis. Analyzing the process of collagenolysis, Sehter and Milanova described a cell very similar to fibroblast, which has the same cell origin, but, is characterised by its double role and a specific function.⁶ Namely, in the cytoplasm of this cell there are numerous cisterns of granular endoplasmatic reticulum (RER), an apparatus for collagenosynthesis and, on the other hand, the presence of great number of primary and secondary lysosomes with collagen fibers in them, in different stadiums of the structure lysis.⁶

The aim of the work

The aim of this article is to show electron microscopically the presence of a cells which

karakteristike izdvajaju iz grupe postojećih čelijskih tipova fibroblastnog čelijskog niza ili su čelijska modulacija fibroblasta.

Materijal i metode

Ispitivanje je izvršeno na jednom slučaju bolesnika muškog pola, starosti 50 godina, koji je bolovao od parodontopatije zadnjih 15 godina, i dijabeta tip II, u vremenskom trajanju od 10 godina.

Posle ekstrakcije prvog donjeg molara sa desne strane, uzet je biopsijski uzorak gingive, koji je fiksirani u osmijum tetraoksidu i obrađeni odgovarajućom standardnom procedurom², a potom posmatrani pod elektronskim mikroskopom JEOL 100.

Rezultati rada

Histološki, u tkivu gingive se svetlosnomikroskopski zapaža gubitak osnovnih karakteristika kolagenih snopova, njihova liza do stvaranja pseudocističnih šupljina, polimorfonuklearni leukociti, limfociti, plazmociti, makrofagi i dosta fibroblasta.

Elektronomikroskopski zapažene su varijante fibroblasta, kao i ćelije koje po spoljašnjoj formi nalikuju fibroblastima i fibroцитима, ali se jedna podvarijanta ovih ćelija ultrastrukturno razlikuje od drugih ćelija fibroblastnog niza po svom organelnom sadržaju. Naime, te ćelije su duguljaste, sa po nekim citoplazmatskim produžetkom. Jedra ovih ćelija su postavljena centralno, nazubljenih su ivica i sa izraženim perifernim heterohromatinom, kao i obiljem euhromatina; na spoljašnjoj membrani jedrove ovojnici nalaze se zgasnuti ribozomi (slika 1). Citoplazma pokazuje neuobičajenost prisustva i rasporeda organela. S jedne strane jedra asimetrično su postavljene nedilatovane i dilatovane cisterne RER-a i slobodni ribozomi. S druge strane, obično su zastupljeni primarni i sekundarni lizozomi, u neuobičajenoj količini za fibroblaste i fibrocite, a koji su intenzivno angażovani u fagocitnoj aktivnosti (slika 2 i 3). U lumenima sekundarnih lizozoma može se videti zrnasti raspad kolagenih vlakana (slika 3).

ultrastructural characteristics distinguish themselves from the group of existing fibroblast cell types.

Materials and methods

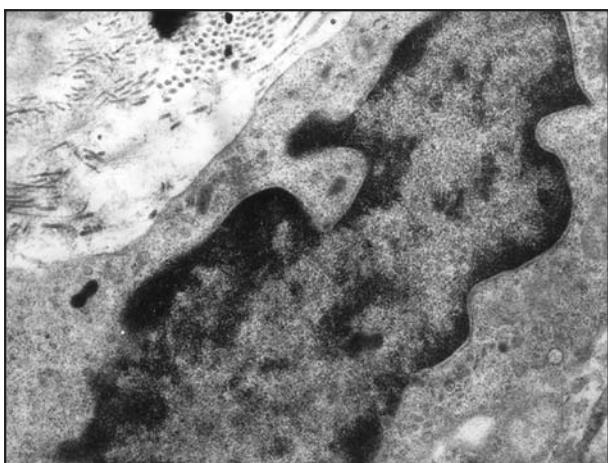
The examination has been carried out on one male patient, 50 years of age, who has been suffering from periodontal disease for 15 years and type II diabetes mellitus, in duration of 10 years.

Biopsy samples of gingiva in the height of 6th teeth of the lower jaw are fixed in 10% puerified formalin, embeded in parafin, and routinely stained with hematoxylin and eosin for light microscopy observation. For the purpose of electron microscopy analysis the material was fixed in osmiumtetroxide and processed by an appropriate procedure, and then examined on EM JEOL 100.

Results

Histologically, in the tissue of gingiva, under lightmicroscope, it is obvious the loss of the general characteristics of collagen bundle, their lysis, and creation of pseudocystic cavities, presence of polymorphonuclear leucocytes, lymphocytes, macrophages, and an abundance of fibroblast like cells.

Electron microscopically, variants of fibroblast are noticed as well as the cells alike fibroblasts and fibrocytes by their general characteristics, but one subvariant of these cells ultrastructurally differs from other cells of fibroblast series in its organel content. Namely, those cells are elongated, with rare cytoplasm extention. Nuclei of these cells occupy central positions, with indented edges, an emphasized peripheral heterochromatin, and centrally abundance of euchromatin; on the outer membrane of nucleus envelope are present numerous ribosomes (Fig. 1). Cytoplasm shows particular presence and arrangement of organelles. Namely, they are asymmetrically located, on one side of the nucleus, non-dilated and dilated cisterns of rough endoplasmatic reticulum and free ribosomes. On the other hand, primary and secondary lysosomes are usually present, in an unusual quantity for fibroblasts and fibrocytes, with intensive engagement in phagocytic activity (Fig. 2, 3). In secondary lysosomes, granular disintegration products of collagen fibers can be seen (Fig. 3).

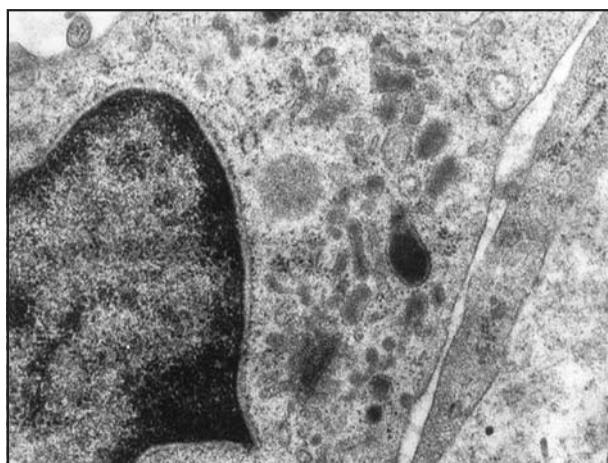


Slika 1. Elektronomikroskopska grafija ćelije duguljastog i nazubljenog, uglavnom euhromatskog jedra, sa dilatovanim cisternama RER (iznad jedra) i grupicama lizozoma (ispod jedra), zapazi prisustvo mnoštva kolagenih vlakana u pericelularnom prostoru.

EM x 6000.

Figure 1. EM-graphu of a cell with longish and cogged edges, mainly euchromatic nucleus with deleted cisterns RER (above the nucleus) and small groups of lysosomes in pericellular space; notice the presence of a great number of collagen fibers in pericellular space.

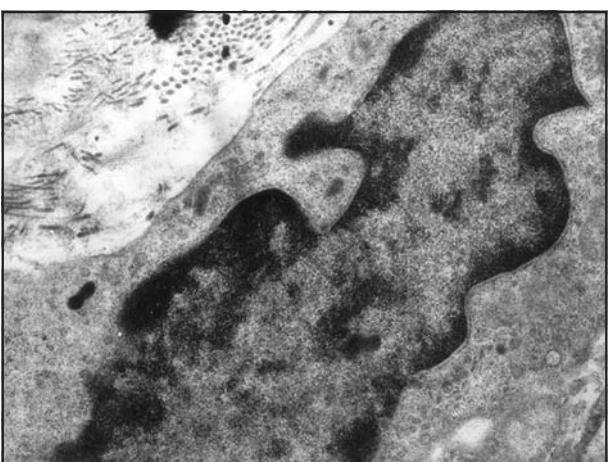
EM x 6000.



Slika 2. Ćelijski produžetak fibroklasta sa slobodnim ribozomima i cisternama RER; jedro je euhromatsko, na spoljašnjoj membrani njegove ovojnica je zgasnuti niz ribozoma, a u citoplazmi brojne cisterne RER-a i mnoštvo lizozoma u različitim fazama funkcije (na osnovu gustine sadržaja).

EM x 6000.

Figure 2. Cellular extention of fibroblast with free ribosomes and cisterns RER; nucleus is euchromatic, on the outer membrane of its involucrum there is a condensed series of ribosomes, and in the cytoplasm numerous cisterns of rough endoplasmatic reticulum (RER) and many lysosomes in different phases of the function (on the basis of density of contents). EM x 6000.



Slika 3. Fibroklasti sa jako uvećanim sekundarnim lizozomima u kojima se naziru kolageni fibrili u razgradnji, a u ostaloj citoplazmi odgovarajuće i jako dilatovane cisterne RER; zapazi pericelularno kolagene fibrile. EM x 6000.

Figure 3. Fibroblasts with very much enlarged secondary lysosomes in which one can hardly perceive collagen fibres in the process of disintegration, and in the other part of cytoplasm corresponding and highly deleted cisterns RER; notice pericellularly collagen fibers. EM x 6000.

Diskusija

Ne ulazeći u pitanja inicijacije i mehanizma odgovornih za održavanje ovog destruktivnog procesa, želimo da istaknemo postojanje žestine kolagenolize kod parodontopatije, naročito dijabetičara. Ovo znači da, pored poremećaja ravnoteže kolagenosinteze i kolagenolize (uz pomoć litičnih enzima), sa prevagom ove druge, ovom procesu pridružuje se i nova ćelija, fibroblast, koja je bogato snabivena lizozomnim aparatom. Fibroblasti direktno učestvuju u resorpciji kolagenih struktura uvlačeći u sebe kolagena vlakna i vršeći

Discussion

Without bringing up the questions of initiation and mechanisms responsible for maintaining this destructive process, we would like to emphasize the severity of collagenolysis in periodontal disease, especially in diabetic patients. This means that, besides the disturbance of the balance of collagen synthesis and collagenolysis (with the help of the lytic enzymes), with prevailing of the second, a new cell take a part this process, that is fibroblast, which is richly supplied with a lysosome apparatus. Fibroblasts directly take part in the resorption of collagen structures taking in them-

njihovu lizu unutar svojih lizozoma, za razliku od makrofaga, za koje se smatra da deluju na kolagen ekstracelularno, a posle njegove odmakle denaturacije, kada je on lišen strukturne organizacije, oni ga fagocituju.^{2,8-12}

Moguće je da fibroblast, kao i fibrocit, luči enzime u okolinu kako bi se održala ravnoteža u procesima geneze i lize kolagena. Međutim, smatramo da se fibroblast pojavljuje kao izraz provokativne ekscesivne kolagenolize i da on potiče iz fibroblastne ćelijske linije, kao fenotipska modulacija, a ne iz makrofagalne ćelijske diferencijacije. Njegovo prisustvo bi moglo da se dovede u analogiju sa pojmom miofibroblasta, koji takođe ima dvostruku ulogu, na mestima gde njega normalno nema, već samo u toku reparatornih procesa (zarastanje rane).¹³ Postoji i mogućnost da je fibroblast indukovana ćelija od stem ćelije fibroblasta, a ne transformisani već postojeći differentovani fibrocit.

selves collagen fibers and carrying out their lysis within their own lysosomes, unlike microphages, that are considered to influence collagen extracellularly, and after its progressive denaturation, when it is deprived from structural organization, they phagocytose it.^{2,8-12}

It is possible, that fibroblast, secretes enzymes into surroundings, in order to maintain the balance in the processes of genesis and lysis of collagen. However, in our opinion, the fibroblast appears as an expression of an excessive, provocative collagenolysis and it comes from fibroblast cellular series as a phenotype modulation, and not from macrophagal cellular differentiation. Its presence could be in analogy with the appearance of myofibroblast, which also has a double role, in the places where it normally is not present, but only during reparative processes (wound healing)¹³. There is also a possibility that a fibroblast is an induced cell from the stem cell of fibroblast, and not transformed but existing differentiated fibrocyte.

Zaključak

Visokoagresivna kolagenoliza gingive i periodoncijuma kod parodontopatije dijabetičara, indukuje razvoj nove ćelije, fibrolasta, koja istovremeno raspolaže sposobnošću kolagensinteze lučenjem proteolitičkih enzima u ekstracelularni matriks i fagocitozom i intracelularnom degradacijom kolagenih vlakana.

Conclusion

Highly aggressive collagenolysis of the gingiva and periodontium in periodontal disease in diabetics, induces the development of a new cell, fibroblast, which, at the same time, has the capability of collagenosynthesis by secreting proteolytic enzymes into extracellular matrix and phagocytosis and intracellular degradation of collagen fibers.

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