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GENERAL OVERVIEW OF THE EPITHELIAL DIFFERENTIATION MECHANISMS AND FEATURES OF THE ORAL MUCOSA MICROCIRCULATION

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Abstract. *Introduction.* Aspects of the morphological structure of the gums are currently attracting the general attention of both domestic and foreign scientists. Interest in this problem is due to the need to identify the aetiology and pathogenesis of numerical disorders in this area, such as chronic catarrhal and chronic hyperplastic gingivitis.

Aim of research. To analyse the data of literature sources for the last 10 years with the characteristics of modern views on the mechanisms of the epithelium differentiation of the oral cavity mucous membrane.

Materials and methods. Review and analysis of scientific and medical literature based on databases such as Scopus, Web of Science, MedLine, PubMed, NCBI, the study of which does not exceed 10 years, including literature reviews and clinical trial results.

Results of research. Features of differentiation of the epithelium cellular composition of the oral cavity mucous membrane provide complex mechanisms in maintaining homeostasis processes, initiate new views on the pathogenesis of inflammatory and inflammatory-dystrophic processes of the oral cavity mucous membrane, in the context of cytotopography of the anatomical area peculiarities, and explain the prevalence of pathological processes of the given localization.

Conclusions. The mechanisms of differentiation of the oral cavity epithelium are closely related to the processes of regeneration, differentiation and desquamation, playing a leading role in ensuring the physiological processes and functions of relevant tissues and in the pathogenesis of diseases of the oral cavity mucous membrane. Therefore, the study of the processes of the epithelium differentiation of the oral cavity mucous membrane with in-depth analysis of cytological features of different anatomical areas in the norm and specific morphofunctional changes in the development of the inflammatory process is a relevant and promising area of research.

Key words: epithelium, gums, tissue basophils, epithelial differentiation.

Узагальнююче уявлення про механізми диференціації епітелію та особливості мікроциркуляції слизової оболонки порожнини рота

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Резюме. *Вступ.* Аспекти морфологічної будови ясен у теперішній час привертають загальну увагу як вітчизняних, так і закордонних вчених. Інтерес до даної проблеми викликаний необхідністю виявлення етіології та патогенезу багаточисельних порушень у даній ділянці, таких як хронічний катаральний та хронічний гіперпластичний гінгівіт.

Мета дослідження. Проаналізувати дані літературних джерел за останні 10 років із характеристикою сучасних поглядів на механізми диференціації епітелію слизової оболонки ротової порожнини.

Матеріали та методи. Огляд та аналіз наукової та медичної літератури на основі баз даних Scopus, Web of Science, MedLine, PubMed, NCBI, вивчення яких не перевищує 10 років, включаючи огляди літератури та результати клінічних випробувань.

Результати досліджень. Особливості диференціації клітинного складу епітелію слизової оболонки порожнини рота забезпечують складні механізми у підтриманні процесів гомеостазу та ініціюють до нових поглядів на патогенез запальних і запально-дистрофічних процесів СОПР, у контексті особливостей цитотопографії відповідної анатомічної ділянки, і пояснює поширеність патологічних процесів даної локалізації.

Висновки. Механізми диференціації епітелію порожнини рота тісно пов'язані з процесами регенерації, диференціації та десквамації і відіграють одну із провідних ролей у забезпеченні фізіологічних процесів та функцій відповідних тканин та у патогенезі захворювань слизової оболонки порожнини рота.

Тому вивчення перебігу процесів диференціації епітелію СОПР із поглибленим аналізом цитологічних особливостей різних анатомічних ділянок в нормі та специфічних морфофункціональних змін за умов розвитку запального процесу є актуальним та перспективним напрямком досліджень.

Ключові слова: епітелій, ясна, тканинні базофіли, диференціація епітелію.

Introduction

Aspects of the oral mucosa and its anatomical components organization, in particular the gums, are currently attracting the general attention of both domestic and foreign scientists. Interest in this problem is caused by the need to clarify and detail the aetiology and pathogenesis of inflammatory and inflammatory-dystrophic diseases in different groups of people [1, 11].

As a chewing mucosa, the gums are constantly subjected to mechanical load in the process of chewing food and the formation of a food lump. The epithelium of the gums protects the underlying connective tissue from the action of microbial factors that can initiate inflammation, as well as provides adaptation to physical activity. Under physiological conditions, the epithelium of the gums acts as a barrier not only to microorganisms, but also to physicochemical and exogenous factors. This process is provided by desquamation of surface layers and compensation due to the subordinate basal and parabasal layers by means of their proliferation [10, 14].

Aim of research

To analyse the data of literature sources for the last 10 years with the characteristics of modern views on the mechanisms of differentiation of the oral mucosa epithelium.

Materials and methods

Review and analysis of scientific and medical literature based on databases such as Scopus, Web of Science, MedLine, PubMed, NCBI, the study of which does not exceed 10 years, including literature reviews and clinical trial results.

Results of research

Anatomically, there are two parts in the gums: free and attached [12]. Histotopographically, there are three zones in the mucous membrane of the free part. The first is the gingival papillae, which are located between the crowns of adjacent teeth. The second is the gingival margin, which zigzag envelops different classes of teeth on the vestibular and lingual sides throughout. The third is adjacent to the cervical surface of the tooth enamel and in physiological conditions has a slight deepening [13, 4].

The cells of the basal layer of the gum epithelium have a faster rate of regeneration than other parts of the oral mucosa. At the same time, only 15% of the gum epithelium is keratinized by means of orthokeratosis, when typical horny scales are formed on its surface. The phenomenon of parakeratosis, in the course of which there is apoptosis of spiny cells and their desquamation is observed, occupies 75% of the entire mucous membrane of the alveolar process. Only 10% of the gum area is covered with multi-layered squamous epithelium without keratinization. It should be noted that according to the literature, the last type of epithelium without a clear boundary passes into the epithelium of the sulcus and the epithelium of the attachment. In contrast to the multi-layered squamous epithelium with keratinization, the thickness of which in the areas of the dental papilla is about 255 μm, the epithelium of the sulcus is represented by only 3-4 layers of cells. The latter are represented by basal cells, which are connected to the basement membrane, spiny and surface cells by means of semi-desmosomes. These cells differ in their ultrastructure from the epitheliocytes of other parts of the gums. In addition, the cytokeratin intermediate filaments of these cells are biochemically different from those of other gingival cells.

This position makes it possible to assert the initial process of keratinization of the epithelium of the gingival sulcus. Due to this process, the intercellular spaces in this epithelium are expanded and occupy about 20% of its volume, and the number of desmosomes that bind epitheliocytes is reduced by 4 times compared to the multi-layered squamous epithelium with keratinization. Due to these features, the epithelium of the gingival sulcus has high permeability and provides transport of substances through it in two directions. Thus, from the saliva and the surface of the mucous membrane is the entry of antigens into the tissues of the internal environment, necessary for adequate stimulation of the immune system. At the same time, a gingival fluid consisting of immunoglobulins, compliment components and antibacterial substances is released from the bloodstream of its own plate [10].

The gums and gingival sulcus are the main source of leukocytes in saliva. According

to the literature, transepithelially in the oral cavity about 3 thousand leukocytes per minute normally migrate. According to other literature sources, the number of the latter in the course of pathology increases by an order of magnitude. Most leukocytes not only remain viable, but also have high phagocytic activity [14].

In addition, in the course of inflammatory processes in the gums, the number of migrating leukocytes increases significantly, due to hemotoxic factors secreted by bacteria. This is the reason for the formation of the mucous membrane barrier against the penetration of bacteria through a relatively thin epithelium without keratinization in the underlying tissues. In this case, epithelial cells secrete cytokines and growth factors, which ensure its differentiation and tissue homeostasis.

Thus, according to the literature, the presence in the mucous membrane of the alveolar process of non-keratinized epithelium with the initial stage of its keratinization creates a special hemato-cellular barrier against the penetration of microorganisms into the underlying connective tissue. The function of this barrier is impaired in inflammatory conditions.

In addition to the above-described mechanism of protection, in the mucous membrane of the alveolar process there is another mechanism due to the presence of the stratum corneum epithelium on the outer surface. It is formed as a result of the differentiation of epithelial cells into horny scales with the simultaneous synthesis of keratohyalin. Various physical and chemical damaging agents acting on the gums determine the protective reaction in the form of thickening and compaction of the stratum corneum, which desquamates in the form of scales together with microorganisms contained in the oral cavity.

The morphological characteristics of keratinization include two interrelated processes: the synthesis of fibrillar elements and their transformation into keratin fibrils, as well as the gradual rearrangement of epithelial cells with the disintegration of the nucleus and intracellular organelles, culminating in the formation of horny scales.

The process of primary keratinization begins in the cells of the basal and prickly layers, by transforming primary tonofilaments into their bundles – tonofibrils; the latter in prickly cells have complex architectonics, and play the role of the intracellular as well as the perinuclear protective-depreciation framework. In granular cells, this skeleton is destroyed and keratohyalin complexes are formed. The latter are synthesized by special structures of keratinosomes - Odlander granules, which are located in the cytoplasm of epitheliocytes of granular and partially spiny layers of the epithelium [6].

It should be noted that the question of the role and mechanism of keratohyalin formation remains open. Although most authors believe that keratohyalin is re-synthesized by keratosomes in granular cells and deposited on the filaments, forming complex tonofibrillary-keratohyalin complexes in the stratum corneum. As they move from the basal layer to the spiny one, cells complicate their structure in the form of the tonofibrillary system and the specialization of the mechanisms of intercellular desmosomal connections. However, from the granular layer to the appearance of the stratum corneum, the cells enter a stage of regression. There is an expansion of intercellular spaces and the destruction of desmosomes, as well as gradual karyopyknosis with subsequent lysis. Due to necrosis, non-nuclear horny scales are formed in the stratum corneum of the epithelium, which under physiological conditions are desquamated together with microorganisms.

A slightly different process of keratinization occurs in the gums, where the type of keratinization is represented by parakeratosis. Thus, on the surface of the epithelium, there are flat cells that contain keratin, but they include pyknotic nuclei, slightly reduced in volume due to genetically programmed necrosis - apoptosis. In this case, around the nuclei there are apoptotic bodies, as well as the remains of organelles in the vacuolated cytoplasm. In the surface granular layer, single keratohyalin granules can be found, and in parabasal cells - keratohyalin inclusions. Intercellular spaces are expanded, desmosomes are visualized. Given this histostructure of the epithelium, some authors suggest that in the course of parakeratosis the mucous membrane has the ability to reabsorb.

Thus, the mucous membrane of the alveolar process has different properties to keratinization, which determines its special functional barrier - in physiological conditions, along with the peculiarities of the microcirculatory tract.

The blood circulation in the gums is more intense than in other anatomical areas of the oral mucosa, which is due to functional features. It is provided by arteries that run parallel to the surface of the mucous membrane, and give

vertical branches that penetrate in the reticular layer of connective tissue, forming extensive capillary plexuses in the papillary layer. The shape of the capillary loops is determined by the shape of the papillae, and their number - by the volume of the papillae. From the postcapillaries, blood enters the venules, which are located near the arterioles. Between them there are different types of arteriolovenular anastomoses that regulate the blood supply to the gums under physiological conditions.

Arteriolovenular anastomoses are connections of vessels that carry arterial blood rich in nutrients and oxygen bypassing the capillary bed, regulating blood flow to the mucous membranes of the gums. The volume of blood circulation in arteriolovenular anastomoses is quite intense due to the fact that their diameter varies from 30 to 500 μm. At the same time the speed of blood circulation is considerably increased.

Thus, if 1 ml of blood passes through the capillary within 6 hours, the same volume of blood passes through the arteriolovenular anastomosis in 2 seconds.

There are two groups of anastomoses. In the first group of true arteriolovenular anastomoses, arterial blood is shunted, and the filling is regulated by the smooth muscle cells of the middle shell of the arteriole itself. In the second group of arteriolovenular anastomoses, the regulation of blood circulation is carried out by special contractile structures located in the lumen of the shunt, while mixed arteriovenous blood is ejected. It should be noted that arteriolovenular anastomoses have a high reactive property to external stimuli and rhythmically contract with a frequency of 12 times per minute. However, the frequency of their reduction in the mucosa of the alveolar process may change during the act of chewing [7].

However, according to the literature, there are three main mechanisms of blood vessels regulation: neuromuscular, neuroparacrine and endothelium-dependent.

In the neuromuscular mechanism, the coordinated response of vessels is realized through the efferent chain, which includes monoaminergic and cholinergic axons. A larger part of axons form distant effectors, and a smaller one form typical neuromuscular synapses with smooth muscle cells, mainly arterioles. Some axons contain dense vesicles with norepinephrine, other axons are filled with acetylcholine.

According to the literature, the features of the microcirculatory tract in the norm of various anatomical parts of the oral cavity, including the gums, have been studied very carefully. However, it is of practical interest what morphological changes take place at the level of the microcirculatory tract of inflammatory processes and inflammatory-dystrophic processes. This in turn is of practical importance for the application of pathogenetic and symptomatic treatment.

The neuroparacrine mechanism of the blood vessels regulation is carried out by tissue basophils.

The release of inflammatory mediators - biologically active substances - is considered as a trigger of inflammation, which determines the entire subsequent picture of the inflammatory reaction. Tissue basophils play a leading role in the process of isolating inflammatory mediators.

There are various classifications of tissue basophils in the literature. Akin C. [2] subdivides tissue basophils into small cells with orthochromatic granularity, located in the adventitia of blood vessels (devastated); larger cells with abundant metachromatic granularity, located along the capillaries and in the intercapillary space; mast cells with a pronounced phenomenon of degranulation.

According to Brockow K. [5] tissue basophils are subdivided depending on the stages of secretion synthesis. The first stage - tissue basophils are close to large lymphocytes. In the cytoplasm, there are many Schick-positive granules and a lot of alcian-positive grains, coloured blue (high activity of proteolytic enzymes). At the second stage, part of the granules of tissue basophils is stained with alcian blue, as well as with safranin red, which gives a Schick-positive reaction; the nuclei are spherical, their volumes are reduced, chromatin is accumulated. The third stage - the cytoplasm is filled with safranin-positive granules. There is a direct reaction of the granules in Schick colouring (completion stage of the granules' skeleton formation). The fourth stage - large tissue basophils, stained brick-red when stained with alcian blue and safranin. There is a positive colour for histamine with parabromaniline.

According to the works of Moharamzadeh K, Colley H, Murdoch C et al. [12] tissue basophils are subdivided, depending on the stages of differentiation, into young, maturing, and mature forms. Young forms contain small gamma-metachromatic granules on the periphery of the cytoplasm, and the perinuclear zone is free, due

to a large number of mitochondria. This type of tissue basophils is localized in the surface layer of the dermis near the basement membrane. Maturing forms of tissue basophils are located along the capillaries; there is beta-metachromasia in their granules. Mature forms of tissue basophils are characterized by orthochromasia of granules and pronounced degranulation.

The secretory process in tissue basophils is associated with the synthesis, accumulation, and excretion of biologically active substances (mediators) found in secretory granules. The latter contain electron microscopic formations in the form of laminar (twisted plates), crystal-like structures with fine- or coarse-grained matrix. In some organs, tissue basophils may be dominated by granules of a certain structure that correspond to the specifics of their biochemical composition. Thus, the presence of laminar structures indicates the presence of heparin. According to electron microscopic and immunocytochemical studies, such a neutral protease as chymase is localized mainly in dense amorphous areas of granules, and tryptase - in less dense areas containing crystals [11].

There are three types of secretion of tissue basophils: apo-, mero- and holocrine: apocrine secretion - the release of granules by means of exocytosis with cell preservation; merocrine secretion - the appearance of light vacuoles in the cell and metachromatic halos around the cell. The substance of the granules dissolves in the perigranular space (granulolysis), and then penetrates through the intact cell membrane into the intercellular substance; holocrine secretion - the release of the entire mass of granules and cell death.

Apocrine secretion in the form of exocytosis is developed very quickly after the anaphylactic activation of tissue basophils and is manifested by loosening and swelling of the granules. The latter are connected to the degranulation pores of the outer cell membrane. Hemispherical protrusions are found on the surface of tissue basophils, which are separated from it by fragments of the membrane into the intercellular space [1, 11].

Merocrine secretion is characterized by gradual degranulation of tissue basophils, by means of microvesicular transport, without destruction of the plasmolemma.

In the course of the holocrine type of granules secretion, membranes and the contents of the channels are removed from the cell, which becomes immature and is characterized by a high content of arginine in the nucleus, and gives a direct Schick reaction.

There is no doubt that the mechanism of the granules secretion by tissue basophils is due to the specific biochemical composition of biologically active substances involved in maintaining tissue homeostasis. The main substances that are accumulated in the secretory granules of tissue basophils are histamine, proteoglycans, and neutral proteases.

Histamine is the only representative of biogenic amines in granules of human tissue basophils. It causes pathological manifestations associated with an anaphylactic reaction due to the presence of H1-, H2- and H3-receptors in connective tissue cells. Thus, due to the presence of H1-receptors, there is a reduction in vascular leiomyocytuses and the release of monocytes. H2-receptors in glandulocytes provide mucus secretion, activation of endothelial cells, and the release of neutrophilic leukocytes. Due to the presence of H3-histamine receptors, autoreception of tissue basophils is carried out [9].

Proteoglycans in the granules of human tissue basophils are represented by heparin and chondroitin sulphate E. Their main intracellular function is to ensure the location of synthesized products in granules by blockade of proteases; at the same time, heparin in addition to the known anticoagulant effect has immunomodulatory and antiallergic properties [3].

Neutral proteases include tryptase, chymase, cathepsin. By cleaving fibronectin and increasing the permeability of the wall of the microcirculatory tract, tryptase can promote the migration of tissue basophils in the tissue.

Because of this, determining the level of tryptase in the blood and its blocking can serve as a new direction in the treatment of many allergic diseases. The role of chymase, which destroys the basement membrane of the epithelium, breaks down neuropeptides of the diffuse endocrine system cells and stimulates the secretory activity of the glands, is determined somewhat differently.

Mediators released from tissue basophils are divided into two groups: precursors (histamine, serotonin, eosinophilic chemotactic factor), and newly formed (slow-reacting anaphylaxis substance, prostaglandins, platelet-activating factor, substance contracting smooth muscles). The former are released non-cytotoxicly, the latter are formed in already sensitized cells and are

released by interaction with the antigen and the devastation of the cytoplasm of tissue basophils.

Thus, as the analysis of the literature shows, the study of structural and functional features of tissue basophils opens previously unknown prospects for their targeted impact on the development and course of inflammation, in particular, of chronic gingivitis in the acute stage [8].

A significant achievement of modern theoretical morphology is the formation of the concept of the diffuse endocrine system, which takes an active part in maintaining homeostasis of the digestive system epithelium, through the release of biologically active substances - mediators.

The mediators synthesized by cells of the diffuse endocrine system by biochemical properties belong to Amine Precursore Uptake and Dekarboxilation, as they have a pronounced monoaminergic type of metabolism, which characterizes the main feature of such cells - the ability to accumulate precursors of biogenic amines, decarboxylate them and produce biogenic amines or polypeptide hormones. Due to the widespread use of electron microscopy and immunocytochemistry, significant progress has been made in the study of cells of the diffuse endocrine system, which can be discussed according to accepted international classifications.

S-, P-, D1-cells of the endocrine system were found in the gums. S-cells contain polymorphic secretory granules up to 200 nm in diameter, filled with osmiophilic electron-dense substance. There is no light disk between the contents of the granules and its membrane. The bulk of the granules is concentrated in the basal part of the cytoplasm. In addition to serotonin, these cells synthesize another hormone - melatonin. Serotonin stimulates the secretion of mucus by bacterial cells, but it is an antagonist of histamine and blocks chymotrypsin. Under conditions of inflammation, it causes dilation of arterioles, reduction of myocytes in the venule wall, causes venous stasis. Melatonin is a universal regulator of changes in the body associated with changes in circadian rhythms.

P-cells are saturated with relatively small neurosecretory granules of oval shape with a diameter of 120 nm, filled with osmiophilic electron-dense substance. They contain localized bombesin and dopamine. P-cells correspond to Merkel cells, which are associated with afferent nerve fibres and perform receptor function. Their body's branchings are connected by desmosomes with epitheliocytes of the basal layer. In the basal part of the cell, there are neurosecretory granules, which during mechanical deformation of the branchings are released into the synaptic cleft and then transported by axo-plasma current.

Dopamine is a mediator of pain. In some areas of the mucous membrane there are clusters of Merkel cells that are not innervated. In such cases, the substance secreted from P-cells is released into its own plate, which can activate tissue basophils with the release of classical mediators of inflammation [15].

D1-cells synthesize a vasoactive interstitial peptide that has a vasodilating effect. It reduces the tone of vascular smooth muscle caused by the action of histamine, kallikrein, and prostaglandin F2, improves lung ventilation, stimulates chemoreceptors. D1-cells are filled with small neurosecretory granules without a clear membrane.

Conclusions

Analytical review of the literature shows that the mechanisms of epithelial differentiation and features of gum microcirculation are closely related to the processes of regeneration, differentiation and desquamation, playing a leading role in ensuring physiological processes and functions of relevant tissues as well as in the pathogenesis of diseases of this anatomical location. Therefore, the study of the processes of epithelial differentiation with in-depth analysis of cytological features of different anatomical areas in the norm and specific morphofunctional changes in the development of the inflammatory process is a relevant and promising area of research.

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