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REACTIONARY VERSUS REPARATIVE DENTINE IN DEEP CARIES

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ABSTRACT

The dentine-pulp complex response in deep caries is histological characterized by tertiary formation and mild chronic pulp inflammation. The quiescent primary odontoblasts are reactivated, laying down reactionary tertiary dentine. In more severe carious damage the primary odontoblasts die and reparative tertiary dentine is secreted by odontoblast-like cells, which are differentiated in adult teeth mainly from dental pulp stem cells DPSC. Though associated with reversible pulpitis DPSC still preserve in deep caries the capability of migration, proliferation and differentiation. Some common mechanisms of molecular signals involved in tertiary dentine formation might also explain the balance between inflammation and regeneration of dentine-pulp complex.

Keywords: deep caries; reactionary dentine; reparative dentine

Introduction

The dentine and pulp tissue are biologically intimately related during odontogenesis and afterward throughout the whole existence of the tooth so that they are termed jointly in dental literature as dentine-pulp complex. However, the dentine morphogenesis, histological structure, physical properties, and functions are ultimately depending on the dental pulp vitality. As soon as the pulp becomes necrotic the dentine stops its continuous process of formation, including secretion and mineralization, based on existence and normal function of some specific pulp cells, namely the odontoblasts (1,2).

During the lifetime of a vital tooth the dentine generation is hierarchically described in three main stages as follows: primary dentine, secondary dentine, and tertiary dentine. Primary dentine is formed prior to tooth eruption as opposed to secondary dentine that vital tooth is the result of a continuous life-long process of deposition. Though having a slower rate of deposition the secondary dentine has the same tubular structure as the primary one. Moreover, the continuity of dentinal tubules is also preserved (3).

The tertiary dentine is quite different, mainly because does not show the same homogenous histological feature compared

to the earlier formed primary and secondary dentine. Additionally its secretion is elicited by important noxious stimuli of the dentine-pulp complex such as dental caries or subsequent current operative procedures aimed at tooth crown restoration. Depending on specific pulp cells involved in tertiary dentine secretion, the dental literature depicts either a reactionary or a reparative tertiary dentine (1-3).

Since currently the pulp biology and vital therapies are completely integrated in the concept of regenerative endodontics, the pivotal approach of modern research in the field of tertiary dentine is the cell-centered principle of dentine formation. Of utmost importance is to know to what extent the tertiary dentine is induced by native primary odontoblasts (reactionary dentine) or by secondary odontoblasts derived from stem cells of different sources (reparative dentine) (4-6).

Native odontoblasts – key cell of dental pulp

The functional odontoblast is differentiated from preodontoblast due to the involvement of various growth factors with paracrine or autocrine role in dental development (GH, IGF-1, IGF-2, TGF-β1, TGF-β2, TGF-β3, BMP-2, BMP-4, BMP-6), and transcription factors (Msx1, Msx2) that regulate a cascade of molecular and cellular events during odontogenesis (7).

Columnar in cell outline in the crown or cuboidal in the root, the odontoblasts are located at the interface of dental pulp with predentine, strengthening the intimate tissue relationship through their outward cellular processes extended in dentinal tubules (1,3).

Their main function is to constantly lay down predentine and to support its transformation in dentine by mineralization. As postmitotic cells the odontoblasts are not able to divide so that in case they dye can not be replaced by other matching cells. Accordingly the predentine secretion ceases (1-3,8).

The odontoblasts provide an anatomical and functional defensive barrier at the periphery of dental pulp that control within the dentine-pulp complex the close relationship between these two tissues. The odontoblasts have a major task in initial response of the dental pulp to external insults and also close the curtain by laying down the tertiary dentine as long as they may survive to injury (1-3,8).

Physiological dentinogenesis

In healthy untreated teeth the physiological dentinogenesis encompasses both primary and secondary dentin, which is secreted by odontoblasts throughout life in parallel with normal aging. Though histological are similar these two kind of dentine are separated by the discrete calciotraumatic line. However, the speed of dentine generation varies having different rates, from 4 μm/day for primary dentine to 0.4 μm/day in case of secondary dentine. Particularly after completion of tooth root the secondary dentine secretion is considerably reduced though in incisors or in cusp area of molars some overcrowding of the odontoblasts may be gradually induced (7).

Pathological dentinogenesis

The pathological stimuli from oral milieu such as tooth decay, attrition, cavity preparation, microleakage, chemical or mechanical irritants are triggering the tertiary dentinogenesis, the last stage of dentine formation. The native odontoblasts, which were the responsible cells in primary and secondary dentinogenesis, are also involved initially in secretion of tertiary dentine. The previous molecular control that downregulated the odontoblasts while passing from primary to secondary dentine secretion is supposed to be reversed in order to stimulate the secretory activity of same cells, the odontoblasts (5,7).

Depending on the damage that these noxious factors are inducing in connective pulp tissue can result two types of tertiary dentine, reactionary and reparative (3). On the whole during the dentine-pulp complex healing by tertiary dentinogenesis the secondary odontoblasts reiterate the function of primary ones (9).

In dentine morphogenesis are involved five major signaling networks as follows: BMP (bone morphogenetic proteins), FGF (fibroblast growth factors), Wnts (wingless-related proteins and int-related proteins), Hhs (hedgehog proteins), and

TNF (tumor necrosis factor) distinct expressed both spatial and temporal by corresponding genes. However, in adult permanent teeth is involved mainly BMP family (BMP-2, BMP-4, BMP-6, BMP-7) (10).

BMP signaling networks function at three levels: intracellular, on cellular membrane, and extracellular. The molecular signals are transduced from cell membrane to nucleus by various Smad-interacting proteins (9).

The reactivation of formerly quiescent primary odontoblasts and the differentiation of odontoblasts-like cells are regulated by cellular and molecular signaling. Nevertheless the odontoblasts transcriptom changes simultaneously with cell maturation. This issue was proved by severe decrease of p38 transcript during the conversion from primary to secondary dentinogenesis (9,11).

If not identical, despite the death of some odontoblasts, the reactionary dentine is pretty similar in tubular appearance to primary and secondary dentine. Particularly it displays a focal formation. The surviving odontoblasts recover are still vital and preserve their function (1,7).

Though in adult caries free tooth the rate of secondary dentine secretion is extremely low, in case of external insult of a progressing caries process the native odontoblasts are capable to leave their quiescent status and upregulate the basic function by laying down the reactionary dentine.

However, this type of dentine may have a reduced number of tubules and its histological structure sometimes exhibits mild irregularities. Though preserving the hard tissue continuity, the trajectory of its own tubules shows sometimes an angle with the correspondent tubules of secondary dentine. The reactionary dentine lies down if dental caries is slowly progressing toward the pulp and its thickness depends on the period of noxious stimuli activity but the rate of secretion is increased compared to secondary dentine (1,7).

The reparative dentine is changing the histological characteristic becoming more irregular and less tubular. Commonly the few existing tubules are not displayed in continuity with correspondent tubules of secondary dentine. The reparative dentine is rather a calcified tissue than a proper dentine since is not secreted by primary odontoblasts that died due to the aggressively external stimuli but by odontoblast-like cells, also termed secondary odontoblasts (1,7).

Dentinal aging process

Currently the translucent dentine and dead tracts express the dentinal markers of aging (1).

Translucent (sclerotic) dentine is the consequence of a long-lasting peri- and intra-tubular physiological process of mineral lay down that gradually occludes the dentinal tubules. Its development with patient aging supposes the existence of still vital odontoblasts. Usually this microscopic type of dentine is encountered beneath processes of severe attrition or slow progressing deep caries and represents an age key marker in forensic dentistry (1-3).

Dead tracts are found in those localized dentinal areas where the odontoblasts already died and subsequently the dentinal tubules became empty. However, at their pulpal extremity these tubules are bordered by tertiary dentine (1-3).

Dentine-pulp complex characters in deep caries

In deep caries, if the long-standing microbial insult is mild to moderate, the primary odontoblasts may survive and secrete reactionary tertiary dentine as a protective mineral barrier (12). It has to be reminded that the thickness of residual dentine situated between the carious cavity and pulp chamber is of utmost value for pulp protection. When is less than 0.5 mm the microbial communication in deep caries is actually comparable with an exposed pulp (6,7).

As in caries progress the microorganisms simultaneously increase the strength of pulp attack the primary odontoblasts die. Unfortunately they can not be replaced due to the destiny of postmitotic cells. Nevertheless, the tertiary dentine may continue to be laid down by a novel type of similar cells, the odontoblast-like cells, which are recruited to the site of tissue lesion from undifferentiated mesenchymal cells. This type of tertiary dentine is termed reparative dentine and its formation starts at 20-40 days

after the death of primary odontoblasts (8).

Actually the reparative dentine should be considered a scar increasing the thickness of pulp mineralized barrier but not a real healing since as compared to reactionary dentine it is an amorphous structure differentiated by morphological irregularities and less dentinal tubules. This type of dentine has a reduced permeability and sensitivity to external stimuli (7).

The secretion of tertiary reparative dentine depends on the extent and duration of microbial aggression. Permanent inflammatory or degenerative damages of dental pulp are additional factors reducing the quality of new formed dentine. When the rate of lying down is accelerated the quality of dentine is getting worse due to the higher porosity and numerous defects filled by subsequently intruded connective pulp tissue. This initial formed hard tissue is specific to rapidly progressing deep caries and may be termed rather fibrodentine than reparative dentin (8).

The chronic pulpitis that is commonly associated to deep caries might be considered in some limits an inflammatory reparative response. Thus when the clinical evolution of caries is stopped therapeutically there are fulfilled favorable conditions for the odontoblast-like cells to lay down reparative dentine of tubular appearance. Nevertheless, once the microorganisms invade the reparative dentine its secretion is stopped and finally an acute irreversible inflammatory reaction is installed (8).

Odontoblast-like cell as a link of reparative dentin with regenerative procedures of dental pulp.

The new formed odontoblast-like cells located in direct contact with exposed predentine after the death of native odontoblasts are initially cuboidal and free of odontoblast processes, the cellular components which are particularly in charge with dentinal tubules creation in primary and secondary dentine. After becoming functional in first 3 weeks the rate of predentine secretion was 3.5 μm/day. If the pulp insult ceased the secretion slows down distinctly and after 4-5 months stops (1).

The reparative dentine is not as uniform histological structured as the primary and secondary dentine. It may range from resembling regular primary dentine in superficial caries (mild insult) to an inhomogeneous appearance due to high porosity, poor calcification, areas of interglobular dentine, and reduced number of tubules with noticeable irregularities (rapid progressing caries). Surprisingly the reparative dentine was also found in unerupted caries free teeth (1).

The odontoblast-like cells (secondary odontoblasts) may be differentiated from various stem cells niches such as human exfoliated deciduous teeth (SHED), dental pulp (DPSC) or apical papilla (SCAP), which in this manner supply as progenitors the necessity of replacement for died odontoblasts (7,13,14).

Though alike to bone marrow stem cells DPSC (dental pulp stem cells) are multipotent postnatal stem cells that have the highest capacity to generate specific cells of dentine-pulp complex within the well known process of pulp regeneration (13).

Stem cells reside also in the dental pulp of adults and are able to undergo self-renewal and to maintain throughout the whole pulp life their differentiation potential as well as the phenotype of undifferentiated mesenchimal cells (progenitors) (10).

It is thought that the odontoblast-like cells are born from stem cells, formerly termed undifferentiated mesenchimal cells of dental pulp, due to the involvement of host growth factors (TGF-β1, IGF-1, IGF-2, VEGF, BMP-4) released from predentine or by dentine demineralization provoked during the deep caries progress (1).

TGF-β1 (transforming growth factor β1) is involved in cellular differentiation, including both primary odontoblast and odontoblast-like cells differentiation as well as in MAPK pathway regulation. MAPK/ERK pathway is a complex signal transduction system including many signaling mediators. This pathway is implicated in coupling of intracellular responses to the cell surface receptors where are specifically bound the growth factors (7,9).

It was also found that TNF-α (tumor necrosis factor α) participates in odontoblast-like

cell phenotype differentiation by activating the p38 phosphorylation of MAPK/ERK signaling pathway (4,7,9).

p38 phosphorylation is amplified in tertiary dentinogenesis due to stimulation of the odontoblast-like cells by various growth factors. As p38 is a transcription factor, based on location in cellular nucleus of most components, subsequently it also enhances the own nuclear translocation (9).

TNF-α, TGF-β1, and ADM (adrenomedullin) induce similar patterns of p38 activation. In contrast other tissue growth factors isolated from dentin matrix noncollagenous proteins, such as DMP-1 (dentin matrix protein 1), DSP (dentin sialoprotein) or DPP (dentin phosphoprotein), follow another pattern. DMP-1 is a crucial growth factor in dentinogenesis since it is predominantly found in dentine and deeply involved in its mineralization. Actually the dentine mineralization is prompted by specifically bound DMP-1 to type I collagen. The other two dentin matrix noncollagenous proteins, DSP and DMP, are also implicated in local control of biomineralization (7,9,13).

Though weaker than of growth factors or microorganisms, the stimulatory effect of dentin matrix noncollagenous proteins on p38 phosphorylation is more enduring. It has to be highlighted that microorganisms can not directly activate the quiescent odontoblasts. However, the microbial insult may indirectly approach the odontoblasts by interplaying synergistic with growth factors (7,9).

Relying on this intimate relationship of dentin matrix noncollagenous proteins with p38 phosphorylation and the central position of pulp regeneration in regulating the balance inflammation-repair, a very attractive hypothesis suggests that DMP might have a potential role in preventing the irreversible inflammation of pulp tissue (7,9).

Reparative dentine – a window to pulp regeneration?

Though after a conservative approach in deep caries the tooth usually preserves its vitality, any clinical healing illustrates a more or less pronounced decrease of pulp physiological potential due to its anatomical volume reduction induced by reparative dentine formation, pulp fibrosis, and calcifications. Actually the highly expected healing is leading to pulp aging (2,8). After caries removal by an adequate treatment the dental pulp recovery may be considered a restitutio ad functionem rather than a restitutio ad integrum (15).

In addition is also inquiring if the new mineralized junction within the dentine-pulp complex between secondary and reparative dentin is sufficiently protective for the pulp as its morphology, permeability and the reduced number of bordering pulpal dendritic cells are doubtful (1).

However, some studies demonstrated that during caries invasion in secondary dentine the relatively quiescent native odontoblasts are reactivated, laying down reparative tertiary dentine at comparable rates to primary dentine. On the other hand dentinogenesis and dentine-pulp complex healing might have some common mechanisms of molecular signals (7,9,16,17). Accordingly was opened a window between two apparently opposite conditions, reparative dentine and dentine-pulp complex regeneration.

The expectation for regeneration relies upon the persistence of DPSC in deep caries associated with reversible pulpitis, which proved they still preserve the capability of migration, proliferation and differentiation aiming at regeneration of dentine-pulp complex (11).

Moreover, compared to normal pulp, beneath the deep caries it was observed in pulp tissue 8-fold increase of alkaline phosphatase activity and 2-fold expression for 445 genes involved in regenerative mechanisms. This way the cytokine network governing the odontoblast-like function is also activated. Surpisingly, even powerful proinflammatory cytokines such as TNF-α, bacterial lipopolysaccharide, and IL-1β (interleukin-1β) that usually induce stem cell apoptosis proved to stimulate the tertiary dentinogenesis in incipient stages of pulp inflammation. It might be deduced that at least for a while in deep caries may be encountered rather a preinflammatory pulp than an inflamed pulp (11).

Probably a mild pulp inflammation elicited by proinflammatory cytokines facilitates the

recruitment of DPSC in deep caries and subsequent differentiation of secondary odontoblasts capable to generate reparative dentine matrix (11).

It is assumed that similar key secretion pathways of dentine matrix protein, once functional in early differentiated native odontoblasts, might be reactivated later also in mature odontoblasts in case of carious injury (7,16,17-23).

Hence, since in deep caries the pulp inflammation is constantly present, the link between dentine-pulp complex repair and regeneration might be efficiently balanced by both innate and adaptive immune mechanisms (7,24).

Of particular importance would be to found out which of aforementioned pathways might prompt the tertiary dentine secretion instead to continue the common secondary dentinogenesis. This issue is getting also more complex as in tertiary reparative dentine actually are not involved any more native odontoblasts but odontoblast-like cells (7,24).

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