Review Article

Periodontitis: A Hastened and Destructive Inflammatory Induced Senescence

Abstract

Periodontitis is a chronic inflammation of tooth supporting tissues (periodontal ligament, alveolar bone and gingiva). Due to the chronic and intensive inflammation, these tissues undergo accelerated cellular senescence (premature cells cycle arrest) ends up with their degeneration and dramatic changes in the normal aesthetic and architecture of the bone and gingiva of the affected individual. This review is providing insights into the detrimental and inevitable effect of inflammatory cellular senescence during periodontitis.

Keywords: periodontitis; inflammatory senescence; cell cycle arrest.

1. Introduction

Periodontal disease is an inflammatory disease that involves the supporting tissues of teeth and affects almost 90% of the population (1, 2). The main aetiology for causing this disease is bacterial infection in the periodontal region and in mostly associated with a progressively destructive change of the affected structures surrounding the tooth. In severe cases, extreme loss of bone and periodontal ligament supporting the teeth may eventually lead to the loss of these teeth (3, 4).

Usually, cells undergone senescence due to aging, or in reaction cellular damage, toxic environments, or due to cell inability to replicate as it reach the end of its life span (5). Some of the senescent cells are eliminated by the immune system, but in certain circumstances, especially by late life, huge number of these cells would remain and linger that result in the accumulation a sizable proportion of senescent cells in the tissue (6). In general, senescent cells would affect the tissue negatively by releasing molecules that interfere with the normal cellular activities, provoke chronic inflammation, and destroy the extracellular matrix that is essential to tissue properties such as elasticity or load-bearing strength (7). Hence, comprehensive clearance and elimination of senescent cells should produce greater benefits to health as compared to partial or uneven removal of these cells (8).

Senescent cells must be cleared first and then be replaced by other cells that perform healing and regeneration (9). Without new cells migrating into the inflamed or damaged scene, probably the healing process will not take place as usual (10). Protecting or saving the cells from reaching senescence stage (before /during periodontal inflammation) would be a better solution because once these cells reach the senescence stage, they will not be, any more, part of the healed inflamed tissues. Furthermore, naturally establishing a new batch of cells with almost similar architecture to replace the senescent cells is a hard task especially for the exposed or inflamed periodontium with the unique environment of the oral cavity (11, 12).

Rejuvenating or saving periodontal cells from senescence and preserving the normal steps of cells cycle; are crucial factors to prevent the destruction of the tooth supporting tissues. Hence, clinically, senescence can be used as a target to suppress or at least minimize the almost inevitable loss of supporting tissues.

2. Inflammatory induced senescence

It has been reported that gingival fibroblasts were incubated and treated with LPS *in vitro* for 24 and 48 h, to simulate inflammation and induce cellular senescence (13). LPS result in the induction of ROS (14) which damage the gingival fibroblast DNA. Furthermore, DNA damage potentiates the release of pro-inflammatory cytokines, apoptosis and senescence as a factor of p53 status (15). The inflammatory cytokines are main factors in the initiation and maintenance of cellular senescence, and they are also responsible for initiating an innate immune response that eliminates the senescent cells in vivo (16).

It has been reported that fading senescent cells have the ability to produce and secrete various inflammatory cytokines, mediating chemokines and matrix remodelling molecules (7, 17) that detrimentally affect tissue matrix homeostasis and elicit a chronic inflammation. Therefore, individuals especially elderly people are more prone to a number of autoimmune diseases and inflammatory diseases, including periodontitis (18, 19).

In periodontitis, the cells of the periodontium are obviously degraded and cleared by phagocytes, subsequently; this process results in the loss of attachment and alveolar bone. Recent studies have shown that senescent myofibroblasts were limiting the extent of fibrogenesis associated with wound healing during tissue repair (20).

Phagocytic cells activity has an important role in subsiding the inflammation through engulfing and eliminating the apoptotic cells, which subsequently reduce the exposure of tissues to the harmful effect of the inflammatory and immunogenic contents of fading cells (21).

Clearance of the senescent cells is a crucial step to maintain the function and restore the normal un-damaged condition of the tissue. Therefore, tissue dysfunction can be expected in chronic pathological conditions as the senescent cells are not effectively eliminated, leading to their accumulation in the tissue (22), a condition which would further aggravate the inflammation and debilitate the normal surrounding cells (23). It has been concluded that the double-edged sword of senescence is similar to that of inflammation; hence, senescence is beneficial when it is temporary and effectively cleared but pathological if it is chronically lasting and un-cleared (24). These expressions and complexities of senescence would require further investigations to determine the therapeutic pathway, either by increasing or blocking senescence, depending on the context (9, 25). In periodontitis, chronic inflammation causes un-replaceable loss of the damaged periodontium. Hence, blocking of senescence would be one of the important targets in the periodontal therapy (26).

3. Inflammatory senescence and DNA damage response relationship

More than half a century ago, Hayflick and Moorhead concluded that cultured primary human cells have a restricted replication capacity (27). It has been demonstrated that after repeated cell divisions, these cells moved into a permanent cell cycle arrest condition, which is termed replicative or cellular senescence where normal diploid cells are unable to divide (28). Cellular replication induces telomere shortening that ultimately triggers a DNA damage response associated with permanent cell cycle arrest, a condition called replicative senescence (28, 29). It has been stated that senescence is a model-in-miniature of events leading to aging or degenerating and fading of organism (27).

Senescence is driven by intracellular signals which remained unclear until the discovery of telomere erosion and telomerase. Telomeres are made of repetitive nucleotide sequences at each end of a chromosome and protect them from degradation or fusion with neighbouring chromosomes. Telomeres undergo erosion during the division of cell, therefore, telomeres have been used as a “molecular clock” that determines how many times a cell can divide before attaining replicative senescence (30). Telomerase, also called telomere terminal transferase, is an enzyme made of protein and RNA subunits and it adds a specific-dependent to end of telomere (31, 32). Activated telomerase promotes division potential of several types of cultured primary cells, such as fibroblasts (33). Reduced activity of telomerase leads to shortening of telomeres which subsequently lose their protective function (34), this is followed by a DNA damage response (DDR) which stimulates suppressing factors that interrupt the progression of cells cycle, a process which ends up with cellular senescence (35).

Telomere erosion is not the only cause for cellular senescence (6, 36). Other causes that provoke DDR, such as various types of oxidants, gama-irradiation, ultraviolet light, and certain chemotherapies, can also induce senescence (37-39) .

Cellular stress eliciting a DDR can also trigger a programmed cell death which is called apoptosis. Apoptosis is a step ahead to remove the damaged, degraded or pre-neoplastic cells. This will be followed by phagocytosis to clear the apoptotic senescent cells. Senescent cells actively express and secret several types of extracellular modulators such as chemokines, cytokines, and matrix-remodelling enzymes known as senescence-associated secretory phenotype (SASP) (40-42); are also responsible for promoting the clearance of senescent cells by the host immune system or provoke autocrine signalling to sustain the cell senescent state (40, 42, 43).

DDR also leads to the induction of nuclear factor kappa B (NF-kB). NF-kB orchestrates the cell survival pathway, and, together with the coordination of cell-cycle check points and DNA repair,

it enables the cell with limited damage to restore and continue a normal life cycle, unharmed (44). It has been reported that during inflammation or induction by reactive oxygen species, the cells produce a signalling pathways that link DNA damage in the nucleus with activation of NF-κB in the cytoplasm (45). Other studies found that DNA damage-dependent NF-κB stimulation may play an undesirable role in induction of cellular senescence, especially with persistence of DNA damage (46).

4. Effect of inflammatory induced senescence on periodontal tissues healing and cells migration

4.1 Biology of oral periodontal wound healing and cell migration

Hammerle & Giannobile (2014) had carried out a thorough search the literature related to the healing of oral tissues and concluded a "*Consensus Report of Group 1 of the 10th European Workshop on Periodontology*" which stated that "oral soft tissue healing at teeth, implants and the edentulous ridge follows the same phases as skin wound healing" (47). However, the same study recommended that there is a necessity to appropriately outline valid and reproducible pre-clinical models for the assessment of procedures of soft tissue regeneration around teeth and implants. In another study, Hakkinen et al. (2000) concluded that the basic wound healing events of gingival tissues are similar to the healing principles at the tooth-gingiva interphase, especially above the crest of alveolar bone (48).

During evolution, wound healing has become physiologically well preserved due to its crucial importance for survival (48, 49). Many factors and molecules involved in wound healing appear to work and intersect the functions of each other. It has been reported that during wound healing, certain factors responsible for embryonic development are also existed in the granulation tissue (10). Epithelial cells involved in wound healing are found to contain extracellular matrix receptors that are not usually exist in other epithelial cells (50). In addition, special phenotype of fibroblasts are also found in the granulation tissue during healing (51-53).

Wound repair requires the participation of several types of cells, such as macrophages, fibroblasts, and contractile myofibroblasts, during the proliferative phase (54). Myofibroblasts, a specific phenotype of mesenchymal cells, is derived from fibroblasts in the connective tissue and epithelium at the edges of wound, bone marrow fibrocytes, and other nearby transdifferentiating cells circulating in the blood vessels (55, 56). Myofibroblasts play a significant role during wound closure by forming new matrix constituents which remodel the healing tissues (55). Cellular senescence can deleteriously affect the differentiation of

myofibroblasts, hence, it halts fibrosis during wound repair (57). A massive suppression of differentiation of myofibroblasts has been detected in senescent cardiac tissue (58) and in old skin fibroblasts (59). It is sensible to assume that protecting the cells form inflammatory senescence would be beneficial to sustain or promote the healing process at the periodontal wounds.

4.2 Importance of cell migration in periodontal wound healing

Migration of epithelial cells and fibroblasts from the edges of the wound to fill the wound gap is crucial step for re-epithelialization (54, 60-63). Clot formation is the initial response to traumatic injury or surgical procedure. The forming clot protects the opened wound temporarily; and it acts as a scaffold matrix for the epithelial and fibroblasts migration (64). The scaffold matrix is later replaced by a newly formed collagen matrix made by the migrating fibroblasts into the wound gap. Formation of specific ECM molecules by migrating fibroblasts in the wound gap is controlled by vascular endothelial growth factor (VEGF), transforming growth factors-β1 (TGF-β1) and other proteins such as IL-1α, IL-1β, and IL-4 (54). In order to shape and remodel the healing wound area, cells migrating into the wound area are also controlling the proteolysis into the leading edge of epithelium by proteolytic enzymes activated at specific sites of the cell membrane (53, 65, 66).

4.3 Cellular senescence and periodontal wound healing

Differentiation, proliferation, and migration of mesenchymal or stem-like cells to the wound site are vital part of several events to achieve an optimal wound healing (67). Senescence or accelerated cell cycle arrest elicits a damaging effect on the wound healing of oral tissues, including the periodontium and the masticatory mucosa (12, 68). Senescence detrimentally disrupts the three phases of tissue repair, including the transient inflammatory phase, new tissue matrix formation, and tissue remodelling phase. Steps of wound healing result in tissues restoration and prevention of infection and chronic inflammation. Therefore, senescent tissues are very susceptible for bacterial colonization and subsequently inflammatory reactions (11).

Cáceres et al. (2014) found that senescent gingival fibroblasts were not participating in tissue remodeling during wound healing as they had no ability to synthesize actin fibers when compared to the healthy fibroblasts (12). These results suggest that senescence adversely affects normal collagen production and reorganization during wound healing, and halting proper tissue homeostasis and function (11, 12).

It has been reported that expression of collagen 1A1 gene is reduced in the senescent periodontal ligament as the gene promoter has undergone hypermethylation in the senescent cells (69). TGF-β1 expression is crucially affecting the production of collagen (70). Previous experiments have shown that massive loss of collagen in the aged skin is probably due to reduced TGF-β expression together with declined levels of connective tissue growth factor (CTGF) (71). Though the mechanism of TGF-β expression may differ in oral tissues compared to skin (72), it is probable that gingival tissue cells would undergo changes due to the reduced level of TGF-β in the senescent cells. Other molecules involved in the modification and reorganisation of extracellular matrix components are matrix metalloproteinases (MMPs) which regulate the formation of new tissue constituents in gingival wounds (73). Senescent periodontal ligament cells were found to have high levels of MMPs and tissue inhibitors of MMPs (TIMPs) compared with young periodontal cells donors (74). Increased levels of proteolytic enzymes would lead to disruption of tissue repair in senescent tissues as degradation process surpasses reconstruction of new tissue (75, 76).

Previous investigation found that the levels of interleukin-1α (IL-1α) that stimulates plasminogen activator inhibitor-2 (PAI-2) were increased during inflammatory senescence of human dermal fibroblasts (77). Other studies investigated senescent human periodontal ligament fibroblasts have revealed persistent formation of interleukin-6 (IL-6) and tumor necrosis factor–α (TNF-α) which subsequently caused a prolonged inflammation (78). These results showed that chronic inflammatory phase in senescent tissues is a significant factor that delay periodontal wound healing (11).

One of the most important known tissue change resulted from cellular senescence is the reduction of cells capability to migrate (79-83). The limited migration ability is attributed to the structural changes which occur to the cell cytoskeleton in senescent tissues (84, 85). The dynamic actin framework is an important part of the cytoskeleton as it regulates protrusion, adhesions, contraction, and retraction from the cell front to the rear during cell migration (85, 86). Previous studies have shown that vimentin production surpasses action formation in senescent fibroblasts (84, 86, 87). As such, migration deficit is one of the critical properties of senescent cells and has negative impact during wound healing. Since senescent cells tend to secrete cytokines and mediators which provoke the inflammation and degrade the matrix, normal process of wound repair would be further impaired. Another reason for the reduced migration ability of senescent cells is the loss or deficiency of the migratory response to tissue forming factors such as growth factors (88, 89).

CONCLUSION

During periodontitis, cellular senescence affects the implicated tissues adversely, shortens the cell telomere and induces unrepairable DNA damage. It also affects fibroblasts migration, which detrimentally affects the healing of tooth supporting tissue. In severe untreated cases, the inevitable destruction may lead to the loss of affected teeth.

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