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Review Article

Role of integrins in wound repair and its periodontal implications

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ABSTRACT

Wound healing in human periodontium is a complex process which involves both cell-cell and cell-matrix interactions. Integrins play a major role in regulation of these cell-cell, cell-matrix interaction. Wound healing involves two major events i.e. re-epithelialization and connective tissue repair. In this concise review, we will discuss the role of integrins in these major events as well as their implications in periodontal wound repair. Integrins are differentially expressed in both of these major events. In re-epithelialization, keratinocytes express novel integrins receptors αvβ1, α5β1and αvβ6 which are not expressed in normal healthy epithelium. Re-epithelialization also involves interaction of integrins with TGF-β and fibronectin. Similarly, in connective tissue repair, the activation of fibroblast as well as the expression of integrins α5β1 and α3β1 is upregulated. In healthy periodontium, integrin αvβ6 is normally expressed in junctional epithelium which is generally expressed only at wound sites in other parts of the body. The epithelialization at implant surface has not been yet fully explored with respect to interactions among integrins and other extra-cellular matrix molecules.

1. Introduction

Wound healing in human periodonium is a complex process that restores tissue integrity and involves coordinated functions and interactions of different cell types, extracellular matrix, cytokines and certain growth factors.[1] Periodontal wound healing is of great significance in the treatment of periodontal diseases involving non-surgical and surgical procedures.[1] Interactions between cell and extracellular matrix in periodontal wound healing is controlled by certain cell surface receptors including integrins and other ECM molecules.[2]

Integrins are heterodimeric transmembrane glycoproteins receptors formed by non covalently associated α and β subunits and are critical in a number of physiologic and pathologic process including cell differentiation and proliferation in wound healing, cell-cell adhesion, attachment and signal transduction between cell and extracellular matrix.[3,4] Integrins belong to family of cellular adhesion molecules (CAM) and function as cell surface receptors for specific ligands.[5] Adequate ligand binding to both α and β heterodimers is followed by specific cell-extracellular matrix interactions.[5] In mammals, integrin receptor family comprises of 8β and 18α subunits, the combination of which results in 24 distinct integrins having specificity for different ligands.[5,6]

Integrins are expressed by various cell types including epithelial cells, fibroblasts, osteocytes, endothelial cells, leukocytes and platelets.[5] Human epithelial cells express 7 distinct type of integrins α2β1, α3β1, α5β1, α9β1, α6β4, αvβ1, αvβ6.[2] However, due to unique structural morphology, α9β1 and αvβ1 are not reported in junctional epithelium.[2] Interestingly, αvβ6 is absent from most parts of normal healthy epidermis, oral mucosa and is only expressed in junctional epithelium and oral epithelium of gingival papilla.[7,8]

Integrins play a key role in wound repair involving epithelial and connective tissue repair through their function in cell adhesion as bi-directional, allosteric signaling machines.[6,9,10]

2. Epithelial wound healing

2.1. Epithelial integrins and their ligands in wound healing

Certain proteins and glycoproteins act as ligands for epithelial integrins in wound healing. Collagen is seen to be the optimum ligand for α2β1 whereas tenascin binding is also highly expressed in migrating keratinocytes.[11] α3β1 is the receptor capable of binding multiple ligands, however in wound healing, binding of α3β1to laminin 332 (previously called laminin -5) plays a significant role in promotion of migrating pattern of keratinocytes.[2,11] Laminin 332 is highly expressed by migrating keratinocytes and is also seen to be an adequate ligand for α6β4 integrin acting as a nucleator of basement membrane

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organization.[11,12] Integrin α5β1 is of particular significance in wound healing and is key receptor exclusively for serum fibronectin and fibronectin EIII/A.[11,13] α5β1 and αvβ5 are vitronectin receptor and attach to RGD cell attachment sequence of the protein.[14] Integrin αvβ6 is not normally found in healthy epithelium, but appears in wound healing and binds to transforming growth factor β1(TGFβ1) through its latency-activated peptide that contains an Arg-Gly-Asp(RGD) peptide sequence.[2,9,11] Integrin αvβ1, not normally found in healthy epithelium has affinity to attach to fibronectin EIII/A which is believed to be involved in cell interactions with molecules present in the initial clot.[9,11]

2.2. Integrins interactions with extracellular matrix in re-epithelialization

Wound healing involves series of controlled events followed by formation of provisional extracellular matrix, which is mainly composed of fibrin, fibronectin, vitronectin, and migration of epithelial cells from edges of wound.[1] Keratinocytes express novel matrix integrin receptors αvβ1, α5β1and αvβ6 to migrate and adhere to wound matrix proteins that resting kerationcytes in healthy epithelium do not encounter.[10,15] Expression of other β1 intergrins is also upregulated during migration of keratinocytes to wound matrix and is critical in proper re-epithelialization.[9] Integrin α2β1, besides of having crucial role in binding to collagen type I, III and V also induces the expression of MMP1 in keratinocytes, cleave the exposed collagen matrix, and thus facilitate the migration of wound keratinocytes by focalized denaturation of collagen matrix.[9,10] In addition, α2β1 integrin is seen to further facilitate kerationocytes migration by binding to its ligand laminin-332 ϒ2 chain.[16]

Integrin α3β1 is seen to have a complex role in re-epithelialization including the regulation of TGF-β1 mediated responses.[17,18] α3β1 integrins regulates Smad7, which is an inhibitor of TGF-β1 mediated signaling. Smad 7 inhibits TGF-β1 mediated signaling by binding to TGF-β1 receptor by its transient translocation from from nucleus to cytoplasm.[17] Thus, α3β1 integrins may present another mechanism for kerationcytes motility by reducing the attachment of keratinocytes during re-epithelialization.[17] Integrin α3β1 association with urokinase type plasminogen activator receptor mediates focalized activation of plasmin and plasmin mediated digestion of LM-332 which is crucial for keratinocyte invasion to fibrin clot during wound healing.[2,12,13] TGF-β1 role is crucial in re-epithelialization.[17,19] α3β1 in epidermis is seen to regulate angiogenesis by controlling the mitogen-regulated protein 3(MRP3) but its role in oral mucosa is not yet clear.[18]

In migrating epithelial cells α9β1 integrins are highly expressed during early wound repair, considered to be crucial for keratinocyte proliferation at wound edge and are crucial in cell migration through nitric oxide synthase activity which is regulated by Src tyrokinase.[2,10,20,21] Activation of α9β1 integrins upon specific ligation further activates Src tyrosine kinase by phossphorylation of p130Cas and activation of Rac-1.[20] Inhibition of Src Tyrosine kinase results in decreased cell migration activity.[20] α9β1 signaling introduces a novel mechanism for cell migration in which α9 cytoplasmic domain is seen as crucial for coordinated interactions.[20]

Integrin α6β4, ligand for laminin 332, is crucial in formation and stabilization of junctional adhesion complexes called hemidesmosomes and is important in signalling pathways.[9,11] The extracellular domain of integrin α6β4 interacts with laminin-5 and is important in linking the basal keratinocytes to the basement membranee.[2] Integrin α6β4 is necessary for epidermal growth factor (EGF) induced keratinocyte migration which is associated by activation of integrin assoicated tyrosine kinase Fyn and increased phosphorylation of the β4 integrin cytoplasmic domain.[22] Epidermal growth factor promotes disruption of hemidesmosomes, thus increasing cell motility.[22,23] Integrin α6β4 plays key role in reforming basement membrane by binding to prteolytically cleaved α3 subunit of laminin 332, relocating back from wound edge keratinocytes.[2,22]

2.3. Fibronectin receptor integrins and re-epithelialization

Fibronectin acts as an adhesive molecule which plays a key role in binding to integrins as well as other extracellular components including collagen, fibrin, proteoglycans etc.[24]

αvβ1, α5β1and αvβ6 are three fibronectin receptor that binds to RGD sequence of fibronectin, serving as key role in wound healing. These integrins are expressed by keratinocytes after coming in contact with fibronectin-rich provisional matrix.[2] α5β1 expression in keratinocytes occur during early stages of wound healing and promotes keratinocytes adhesion and motility. α5β1 interacts with fibronectin through Ganglioside GM3 and GT1b interactions in which Ganglioside GM3 promotes and GT1b inhibits interaction with fibronectin.[25] α5β1 integrins promotes cell proliferation and limits cell differentiation of non migratory kerationocytes by their interaction with EIIIA(EDA) fibronectin.[2,13] Integrin α5β1 binding to fibronectin seems to induce Epidermal Growth Factor Receptor(EGFR) signaling directly or indirectly which is localized into migrating tip of wound edge, thus serving as key player in both migration and proliferation of keratinocytes during wound repair.[2,10]

Integrin αvβ1, a low affinity fibronectin receptor, is highly expressed by basal epithelial cells against the fibrin clot and at the wound edges and may facilitate keratinocyte migration on the underlying fibronectin EDA by supporting cell attachment without decelerating the migration.[2] TGF-β1 interaction with αvβ1 integrin may help to accumulate and focalize inactive TGF-β1 which are activated later by protelytic cleavage, however TGF-β1 remain inactivated after TGF-β1-αvβ1 integrin interaction.[26]

2.4. Integrin αvβ6 and transforming growth factor β in re-epithelialization

Integrin αvβ6 in wound healing, binds to fibronectin and tenascin and is highly expressed in basal and suprabasal keratinocyte layers after the epithelial sheet formation.[11,21] Integrin αvβ6 locally activates Transforming growth factor β(TGF-β) by conformational changes independent from any proteolytic activity.[23,27] TGF-β1-β3 are active in wound healing, TGF-β1induces recruitment of inflammatory cells,including macrophages, initiation of granultion tissue formation.[2,23,27] TGF −β1 and β3 colocalize with integrin αvβ6 in 7 day old gingival wounds.[19] Integrin αvβ6 activates latent TGF-β1, which is synthesized as latent precursor molecule containing latency-associated peptide(β1-LAP) and latent TGFβ1-binding protein(LTBP1).[2,26] TGF-β1 activation can be both integrin dependent and integrin independent, however αvβ6 integrin mediates TGFβ1 activation by binding to RGD sequence of β1-LAP of the TGFβ1 protein complex that is fixed to ECM by LTBP1.[27]

TGFβ1 inhibits epithelial cell proliferation via up regulation of cyclin dependent kinase inhibitors p15 and p21 and promotes keratinocyte migration by switching the cells from a differentiative to regenerative phenotype.[19,23] TGFβ1 is seen to have major function in regulation of inflammation by inducing anti-inflammatory(AIR) response through its immunosupression action on T-cells and macrophages.[19,26] TGFβ1 overexpression has also been associated with profound wounds, causing excessive ECM accumulation and scar formation, which supports importance of controlling of TGFβ1 activity in successful wound healing.[19] TGF-β3 is seen to have anti-fibrogenic action, hence its prolonged activation by αvβ6 integrin mediated interaction may be important in protection of gingiva from scar formation.[19]

3. Connective tissue repair

Epithelial wound healing is followed by connective tissue repair and plays an important role in restoring function of tissue.[1,15]

3.1. Activation of fibroblasts and granulation tissue formation

Fibroblasts get activated, change their gene expression, start migrating towards wound bed during wound healing to form granulation tissue and other ECM molecules.[28] It is associated with change in microenvironment of extracellular matrix with respect to fibroblasts.[28] In healthy epithelium quiescent fibroblasts express collagen receptor α1β1 and α2β1, binds to collagen and do not show migration pattern.[28] Similarly, α5β1 integrin, a major fibronectin receptor shows adhesion to matrix molecules.[2,24] Fibroblasts downregulates the expression of collagen receptors α1β1, α2β1 and upregulates the expression of fibronectin binding integrins α5β1 and α3β1 to facilitate migration of fibroblasts to clot.[28] Fibroblasts migration towards fibronectin is stimulated by platelet-derived growth factor and TGF-β1.[29] Fibroblasts adhesion to vitronectin(in clot matrix) is mediated by integrins αvβ3 and αvβ5.[15,28] Integrins αvβ3 are responsible for cell adhesion and cell migration. αvβ3 and αvβ5 interactions with EDA fibronectin promotes myofibroblast differentiation by stimulation of TGF-β1 which is crucial for granulation tissue formation.[29]

3.2. Integrins: regulators of cell proliferation and granulation

Integrin α5β1 serves as a mechanoreceptor by mediating signals that regulates cell proliferation and stoppage of migration of fibroblasts.[28] Organization of collagen and mechanical tension mediated from ECM is important factor that modulates DNA synthesis and cell survival.[15] Reduction of mechanical strain causes apoptosis of fibroblasts.[28] TGF-β1 downregulation at later stages of wound healing results in further reduction of stimulatory signals for fibroblasts and ultimately mediates signals that regulate cell survival.[28] In other mechanism, integrin α2β1 binding to monomeric collagen promotes cell proliferation while fibrillar collagen acts as a feedback mechanism downregulating late cell growth during wound repair.[28,29]

4. Integrins and periodontium

Normal junctional epithelium differs from normal healthy epithelium in certain aspects including different expression of integrins and alteration basement membrane proteins.[2] JE has true basement membrane towards toward connective tissue of gingiva and simple ECM towards the enamel termed as EBL and IBL respectively.[2]

Various studies have explored the gingival epithelial cell adhesion around tooth root surface as well as implant-PIE (peri-implant epithelium) surface. Integrin α6β4 binding to laminin 332 is crucial for maintaining hemidesmosome interactions in basement membrane.[30] Interestingly, in junctional epithelium co-localization of integrin α6β4 and laminin 332 is seen suggesting that their interaction is crucial for maintaing JE contact with cementum or enamel.[30] But, the mechanism is poorly known in PIE (peri implant epithelium) however the role of integrins in periodontal pocket formation and periodontitis is still to be known.[2] Mutations in protein kindlin 1, a ligand of β1 kertinocytes result in failure of attachment of junctional epithelium to tooth surface suggesting that inter-hemidesmosomal β1integrin-mediated cell adhesion is crucial to firm adaptation of JE to tooth structure.[2] An experimental study by Oshiro and coworkers claimed that epithelialization around the titanium implant surface can be enhanced by treating the implant surface with calcium chloride. An increased expression of laminin 332 was observed around the implant-PIE (peri-implant epithelium) interface.[31] On the other hand, integrin α5β1 along with fibronectin has been proposed as a regulator in osseointegration promoting the adhesion of osteoblasts at bone/implant surface.[32]

Various signalling pathways are proposed to be involved in the adhesion of gingival epithelial cells to the tooth root surface including TGF-β signalling pathway. A recent study has demonstrated that Smad2 is significantly involved in the adhesion of gingival epithelials via integrin α2 upregulation.[33] TGF-β1 signalling is also involved in the synthesis of gingival fibroblasts via integrin αvβ1-induced latent TGFβ1 activation.[34] Also, TGF-β is significantly involved in the apoptosis of junctional epithelial cells caused by periodontopathic bacteria. It has been reported that *Aggregatibacter actinomycetemcomitans (Aa)* outer membrane protein 29 (Omp29) induces apoptosis of gingival epithelial cells through fibronectin/integrinβ1/FAK (focal adhesion kinase) pathway.[35]

5. Conclusion

In conclusion, integrins are the key regulators in wound healing. Many integrins and ECM molecules collectively regulate re-epithelialization. Much of research has been done, to elucidate the mechanism of integrin action and signalling pathways in wound healing in skin and epithelium. In perspective of periodontology, much of the research in relation to integrins and cell adhesion molecules on tooth and implant surfaces has still to be done which can improve and create the future avenues of better knowing the implant surface epithelialization.

Ethical approval

Ethical approval was not required.

Declaration of conflicts of interest

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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