Existence Of Galactin The Stratum And Dermal Stiffen Of Keloid Tissues

Sameer Khan

Department Of Medical Science Kardan University, Afghanistan

ABSTRACT:-

Background: Keloids are recognized as benign dermal fibro proliferative tumors that result from aberrant wound healing in susceptible individuals. Nevertheless, the etiology and pathophysiology of keloids is not fully elucidated yet. A protein that has been implicated in the wound healing process, conversion of fibroblasts into my fibroblasts and production of ECM components, inflammation, and generation of galactin-glycan complexes, is galectin-1 (Gal-1). However, its Existence as well as its implications in benign dermal fibro proliferative tumors such as keloids, has not been completely elucidated. Therefore, in this study we investigated the Existence and localization of Gal-1 in keloid tissues.

Methods: Seven biopsies from patients diagnosed with keloid were selected. Four normal skin specimens were obtained from patients undergoing aesthetic surgery procedures. To identification of collagen bundles, some histological sections were stained following the Mason's trachoma protocol. The Existence of Gal-1 was assessed by immunofluorescence staining using confocal laser scanning microscopy.

Results: Immunostaining revealed that Gal-1 was present in the uppermost cell layers of the thickened epidermis with a gradient of expression that seems to increase from basal layer to suprabasal layers. Surprisingly, Gal-1 was particularly found outlining the cellular margin and intercellular bridges of the upper-spinouts and granular keratinocytes and was absent in the Stratum keratinocytes of normal adult human skin. Interestingly, in the reticular dermis the Existence of Gal-1 was noticeable in the cytoplasm of elongated fibroblasts that were forming cellular bundles of collagen and along the thick and compact collagen bundles, whereas in the dermis of normal skin, Gal-1 immunoreactivity was weak in some fibroblasts.

Conclusion: Our findings suggest that in keloid tissues Gal-1 would not only be regulating the dermal fibroblast proliferation, ECM production, particularly collagen, and abnormal vascularization, but would also be contributing to the altered stratification and terminal keratinocyte differentiation and importantly, to Stratum Stiffen.

KEYWORDS:-Collagen bundles; dermal stiffen; fibroblasts; galectin-1; keloid

Introduction

Keloids are recognized as benign dermal fibro proliferative tumors that result from aberrant wound healing in susceptible individuals [1-7]. They are caused by trauma, surgery procedures, burns, acne, folliculitis, vaccination, herpes zoster, infection and skin eruptions such as chicken pox [5-9]. Clinically, keloids are defined as scars that extend beyond the edges of the original wound invading adjacent healthy skin and do not regress spontaneously [1-10] the lesions frequently occur on the anterior portion of the chest, shoulders, upper back, arms, cheeks and earlobes [1,2,5-10]. Histopathologically, keloids are defined as inflammatory disorders characterized by exhibiting a thickened dermis containing numerous fibroblasts, abnormal vascularization, increased inflammatory immune cells, abundant hyalinised collagen bundles (keloid collagen) and extracellular matrix (ECM) components including collagen, elastin, fibronectin and proteoglycans such as syndical and vesicant, mainly produced by activated fibroblasts [1-14]. These histological alterations proper of the keloid tissue have been associated with the elevated production of growth factors such as TGF- β , FGF-2, PDGF, EGF, IGF and VEGF which regulate fibroblast proliferation, ECM synthesis and angiogenesis, and pro-inflammatory cytokines including IL-1 α , IL-1 β , IL-6 and TNF α which promote fibroblast activation and consequently an excessive deposition of ECM components [1,2,5-7,9,10,15,16]. This is why most studies on keloids focus on dermis and few on epidermis [4,17]. However, recent studies describe the epidermis of keloids as thickened, primarily due to hyper proliferation [4,7,17] and altered terminal differentiation [18] of keratinocytes,; some of them, indicating that keratinocytes also participate in the regulation of fibroblasts activity and might contribute to keloid pathogenesis [4,7,19]. On the other hand, several studies consider genetic predisposition as an important factor in the keloid formation and progression [2,5,9,20] whilst others deem the role of local mechanical forces as a predisposing factor of keloid formation [9]. Moreover, there is evidence suggesting that epigenetic modifications would be involved in the pathogenesis of keloids by regulation of fibroblasts activation, proliferation and apoptosis, as well as collagen and elastin synthesis [3,20- 22]. Nevertheless, the etiology and pathophysiology of keloids is not fully elucidated yet [3-7,18]. A protein that has been implicated in the wound healing process [23,24], conversion of fibroblasts into my fibroblasts [24-26] and production of ECM components [26], inflammation [27], and generation of galactic-glycan complexes [28-30], is galectin-1 (Gal-1). Gal-1 is a member of the galactic family that has high affinity for N-acetylLactosamine (Laconic) disaccharides in both Nan O-glycan's and is differentially expressed by various normal and pathological tissues [31]. It has been detected in the cell nucleus, cytoplasm and intracellular and extracellular sides of cell membrane, and can be secreted and found in the extracellular space [31]. Gal-1, like others gelatins, participates in signaling pathways and regulates a variety of biological responses including cell growth, cell-cell and cell-matrix adhesion, cell differentiation,

migration, proliferation and survival [31-34] and plays a critical role in inflammation, angiogenesis, and tumor development and progression [27,33,35,36]. In human skin, Gal-1 has been implicated in psoriasis [37,38] and malignant lesions including cutaneous cancer [39], mycosis fungoid [40,41], and melanoma [42], and scarce studies have shown that the expression of Gal-1 is up regulated in keloid tissue [43]. However, the Existence, distribution, and localization of Gal-1 as well as its possible role in benign dermal fibro proliferative tumors such as keloids, have not been completely elucidated. Therefore, we investigated whether Gal-1 is present in keloid tissues. We also sought to determine whether the Existence of Gal-1 could be associated to Stratum.

Materials and Methods

Skin Biopsies Seven biopsies from patients diagnosed clinically and histopathologically (H&E) with keloid were retrieved from the archives of Section of Dermatopathology of SAIB. Their characteristics appear listed in Table 1. They were taken from three females and four males, ranging in age from 17 to 32 years old, with average of 23.5 years old. Four normal skin specimens were obtained from patients undergoing aesthetic surgery procedures. To identification of collagen bundles, some histological sections were stained following the Mason's trachoma protocol. This study was approved by the Ethics Committee of the SAIB and performed according to the Declaration of Helsinki.

Indirect immunofluorescence staining

Paraffin-embedded skin tissue blocks were cut in sections of 4 μ m of thick. Paraffin sections were placed on salinized slides (DakoCytomation, Denmark), dew axed in xylene, dehydrated through graded alcohol series (from 100 to 70%), and then placed in distilled water and equilibrated in phosphate-buffered saline (PBS) for 10 min. Non-specific antibody staining was blocked by incubating sections in PBS containing 2% bovine serum albumin (BSA) and 0.1% Tween 20 for 1 hour at room temperature (RT). The sections were incubated overnight at 4° C in a moist chamber with rabbit polyclonal anti-human Galectin-1 antibody (Santa Cruz Biotechnology Inc. Santa Cruz, CA). Sections were washed extensively in PBS and incubated in the dark for 30 min with anti-rabbit Alexa Fluor 594 (Molecular Probes, Life Technologies, USA) at RT, followed by 15 min incubation with DAPI (4',6-Diamidino-2-phenylindole) (Molecular Probes, Life Technologies, USA) for nuclear staining. Finally, the sections were washed in PBS and cover slipped with mounting medium (IMMU-MOUNT, Shandon, Pittsburg, PA). For double antibody staining for Gal-1 and α -smooth muscle actin (α -SM actin), the sections were incubated sequentially with the first primary antibody (anti-Gal-1) in a moist chamber over night at 4° C, washed in PBS and incubated for 30 min at RT with anti-mouse Alexa Fluor 592 (Molecular Probes). The sections were then washed

extensively in PBS and incubated for 4 hours at RT with second primary antibody (mouse ascites monoclonal anti- α -SM actin (DakoCytomation), washed in PBS and incubated for 30 min at RT with anti-mouse Alexa Fluor 488 (Molecular Probes). Sections were washed extensively in PBS and followed by 15 min incubation with DAPI (Molecular Probes), washed in PBS and coverslipped with mounting medium (IMMU-MOUNT). A sample of lung fibrosis obtained during autopsy was used as positive control for Gal-1. All images were captured using a 1×81 Olympus inverted microscope with the Fluo View Confocal Laser Scanning configuration (CLSM) (Olympus America) equipped with software program FV10.ASW version 02.01.01.04 (Olympus Corporation). Image J software (NHI, Washington DC) was used for processing of contrast and brightness.

Results Histopathological analysis of keloid tissues showed reduced rete ridges, Stratum Stiffen with keratinocytes organized as cohesive groups maintained together by means of intercellular bridges, hyperkeratosis, and spongiosis (Fig. 1). Important dermal modifications such as Existence of thin collagen bundles parallel to the epidermis, elevated number of elongated fibroblasts, micro vessels, accumulation of inflammatory cells around the microvasculature, nodules containing elongated fibroblasts attached to randomly oriented collagen fibers encompassed by thin collagen fibers and micro vessels, and coexistence of thick, compact hyalinised collagen bundles with elongated fibroblasts attached, were observed (Fig. 1). Mason's trachoma staining was also used to identify collagen bundles. Staining showed thin collagen bundles randomly organized, mainly located in the papillary dermis and in the upper reticular dermis (Fig. 2A). These thin collagen bundles seem to form cellular bundles of collagen that possibly coalesce into thick and compact collagen bundles in the reticular dermis (Fig. 2B). Staining also revealed the Existence of numerous blood and lymphatic micro vessels, inflammatory immune cells localized perivascular and dispersed between thin collagen bundles, and some thin and thick collagen bundles focally fragmented distributed throughout the dermis (Fig. 2A,C). In vivo Gal-1 immunolocalization To investigate the Existence and localization of Gal-1 in the keloid tissues, dew axed sections were examined by immunofluorescence staining. Immunostaining analyzed by CLSM revealed that Gal-1 was present in the uppermost cell layers of the thickened epidermis with a gradient of expression that seems to increase from basal layer to suprabasal layers (Fig. 3A, 4A). Surprisingly, Gal-1 was particularly found outlining the cellular margin and intercellular bridges of the upper-spinouts and granular keratinocytes in a punctate and linear array (Fig. 3A) and was absent in the Stratum keratinocytes of normal adult human skin (Fig. 3B). Immunofluorescence analysis also revealed the Existence of Gal-1 in the basement membrane and in the thickened dermis of keloid tissues (Fig. 3A). In the later, this protein was detected in the cytoplasm of many elongated fibroblasts that were attached to thin collagen bundles and localized in the papillary dermis and in the nodules of the upper reticular dermis (Fig. 3C,E). Additionally, strong Gal1 immunoreactivity was also detected in some perivascular inflammatory

immune cells (Fig. 3C,D, 4A). Interestingly, in the reticular dermis the Existence of Gal-1 was noticeable in the cytoplasm of elongated fibroblasts that were forming cellular bundles of hyalinised collagen and along the thick and compact hyalinised collagen bundles (Fig. 3F,G, 4B,C), whereas in the dermis of normal skin, Gal-1 immunoreactivity was weak in some fibroblasts (Fig. 3H). There is controversy not only regarding the Existence of my fibroblasts in keloid tissues but also regarding the conversion of fibroblasts into my fibroblasts by Gal-1 [24,26,45]; therefore, we sought to determine by double-immunofluorescence staining whether α -SM actin, the most common marker for my fibroblasts identification [46], was present in the fibroblasts of keloid tissues examined. Immunolocalization demonstrated that α -SM actin was completely absent in the fibroblasts expressing Gal-1 as well as in those that did not it (Fig. 3C,D, 4A,C). Interestingly, double immunostaining also showed a strong immunoreactivity for Gal-1 and α -SM actin in both blood and lymphatic micro vessels in the dermis of keloid tissues (Fig. 3C,D, 4A,C), whereas in the dermis of normal skin, moderate cytoplasmic expression of Gal-1 was observed in both blood and lymphatic micro vessels and some fibroblasts. Additionally, strong immunoreactivity for Gal1 was detected in pulmonary artery branch and bronchiolar epithelial cells when lung fibrosis was used as positive control (Fig. 5), and no immunoreactivity for Gal-1 was detected when the primary antibody was omitted or replaced with PBS (not shown).

Discussion In the current study, we evidenced the Existence of Gal-1 in the Stratum and dermal Stiffen of keloid tissues. Immunolocalization showed strong Gal-1 immunoreactivity in the upper-spinouts and granular keratinocytes with a gradient of expression that seems to increase from the basal layer to suprabasal layers. This particular localization and distribution of Gal-1 suggests its involvement in later stages of keratinocyte differentiation. It is important to highlight that in the keratinocytes from normal adult skin, Gal-1 was not evidenced. Remarkably, previous studies in human skin have shown that Gal-1 is not expressed by keratinocytes in normal skin [37] but can be expressed by cells in psoriasis [37,38], cutaneous malignant lesions including squamous cell carcinoma [39], mycosis fungoid [40,41], and melanoma [42], while in keloid tissues, proteome analysis of keloid proteins performed by Ong et al. revealed that the expression of Gal-1 is up regulated compared to normal skin [43]. However, the role of Gal-1 in skin lesions is still unclear. In this respect, recent in vitro studies have shown that Gal-1 and Gal-3 secreted by malignant T cells regulates proliferation of keratinocytes and induce disorganization of the outer layers of the epidermis, accompanied by decreased expression of the terminal differentiation marker involucrin [47]. Similarly, studies related to gelatins expression and function have shown a differential pattern of Gal-1 and Gal-3 expression during the course of wound healing with Gal-1 being up regulated at the early stages of healing and Gal-3 at the later stages [23,48,49]. Of particular interest, is that increased Stratum thickness not attributable to hyper proliferation, but possibly caused by abnormal terminal differentiation of keratinocytes that involves an increased

expression of involucrin in the spinous and granular layers, has been recently reported in keloid scars [18]. Regarding the expression of Gal-1 in normal and pathological tissues, some studies highlighted that Gal-1 is detected in the cell nucleus, cytoplasm and intracellular and extracellular sides of cell membrane regulating cell growth, cell-cell and cell-matrix adhesion, cell proliferation, differentiation, migration and survival [31-34], and that its expression might be induced by elevated levels of pro-inflammatory cytokines and growth factors [27,33,50,51]. Therefore, it is possible that Gal-1 contributes to the abnormal stratification and differentiation of keratinocytes in keloid tissues by up-regulation of involucrin expression, and that a differential regulation of Gal-1 and Gal-3 expression would be occurring during the progression of keloid considering that Gal-1 was reduced or absent in normal epidermis and was present in the stratum corneum of keloids, while Gal-3 appears gradually in normal epidermis and absent or decreased in the stratum corneum of some skin lesions such as psoriasis, basal cellular carcinoma and lichen planus. Also, it is possible that some pro-inflammatory cytokines and growth factors released from keratinocytes and/or immune cells could be inducing Gal-1 expression. In this study, immunofluorescence also evidenced an augmented intracellular immunoreactivity for Gal-1 in many elongated fibroblasts and few immune cells in the dermal stratum corneum of keloids. We suggest that this increased immunoreactivity for Gal-1 in the fibroblasts could be induced by certain mediators that are released from inflammatory and endothelial cells at the inflammation sites, and that Gal-1 in turn, would induce fibroblasts proliferation and ECM production considering that keloids are defined as chronic inflammatory disease characterized by the existence of numerous fibroblasts, abnormal vascularization and increased inflammatory immune cells [54]. In support of this, studies in animal models of fibrosis have provided evidence that Gal-1 stimulates the fibroblasts. This work was supported by Service Autonomy Institute de Biomedicine (SAIB). Disclosure the authors disclose that they have no conflict of interest. Acknowledgments We thank to Dr. Oscar Reyes for his assistance.

References

- [1] Butler PD, Longaker MT, Yang GP. Current progress in keloid research and treatment. *J Am Coll Surg* 2008; 206:731-741.
- [2] Bran GM, Goessler UR, Hormann K, Riedel F, Sadick H. Keloids: current concepts of pathogenesis. *Int J Mol Med* 2009; 24:283-293.
- [3] Russell SB, Russell JD, Trupin KM, Gayden AE, Opalenik SR, Nanney LB, Broquist AH, Raju L, Williams SM. Epigenetically altered wound healing in keloid fibroblasts. *J Invest Dermatol* 2010; 130:2489-2496.

[4] Jumper N, Paus R, Bayat A. Functional histopathology of keloid disease. *Histol Histopathol* 2015; 30:1033-1057.

[5] Trace AP, Enos CW, Mantel A, Harvey VM. Keloidis and hypertrophic scars: A spectrum of clinical .