Review

Stromal microenvironment in type VII collagen-deficient skin: The ground for squamous cell carcinoma development

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Abstract

Recessive dystrophic epidermolysis bullosa (RDEB) is a skin fragility disease caused by mutations that affect the function and/or the amount of type VII collagen (C7), the major component of anchoring fibrils. Hallmarks of RDEB are unremitting blistering and chronic wounds leading to tissue fibrosis and scarring. Nearly all patients with severe RDEB develop highly metastatic squamous cell carcinomas (SCC) which are the main cause of death. Accumulating evidence from a murine RDEB model and human RDEB cells demonstrates that lack of C7 also directly alters the wound healing process. Non-healing RDEB wounds are characterized by increased inflammation, high transforming growth factor-β1 (TGF-β1) levels and activity, and are heavily populated by myofibroblasts responsible for enhanced fibrogenesis and matrix stiffness. These changes make the RDEB stroma a microenvironment prone to cancer initiation, where cells with features of cancer-associated fibroblasts are found. Here, we discuss recent knowledge on microenvironment alterations in RDEB, highlighting possible therapeutic targets to prevent and/or delay fibrosis and SCC development.

Introduction

Recessive dystrophic epidermolysis bullosa (RDEB) is a highly disabling genodermatosis characterized by skin and mucosal fragility and blistering [1]. Patients with RDEB carry mutations in the *COL7A1* gene encoding for type VII collagen (C7), the main component of anchoring fibrils (AF) [2], microstructures responsible for the anchorage of the epidermis to the underlying dermis. Loss or altered function of C7 makes the skin more susceptible to frictional damage that causes tissue separation within the uppermost dermis. C7 deficiency also causes altered wound healing, which ends up with fibrosis and scarring [3] (Fig. 1). In the most disabling RDEB form, the severe generalized subtype characterized by a complete C7 loss-of-function, the repeated cycle of wound and repair leads to the mitten deformities of hands and feet (pseudosyndactyly) and, in time, to the development of aggressive cutaneous squamous cell carcinomas (SCC) in nearly all patients [1,4]. The risk for cutaneous SCC in RDEB begins during the teenage years and increases thereafter, with a cumulative risk of >90% by the age of 55, and death from metastases in 80% of cases within 5 years from diagnosis of the first primary tumor [4]. An increased susceptibility to SCC is also present in other generalized RDEB subtypes [4,5].

The mechanisms through which C7 lack results in a high risk for cutaneous SCCs are under deep investigation. One of the main contributors to epithelial tumorigenesis is undoubtedly the accumulation of genetic and epigenetic hits that drive cells towards the acquisition of capacities (the hallmarks of cancer as defined by Hanahan and Weinberg) [6] needed for their malignant transformation. However, the altered interplay between the cell component and the extracellular environment is crucial as well, and allows hallmarks of cancer to emerge. Interestingly, the genetic and epigenetic lesions in RDEB-associated

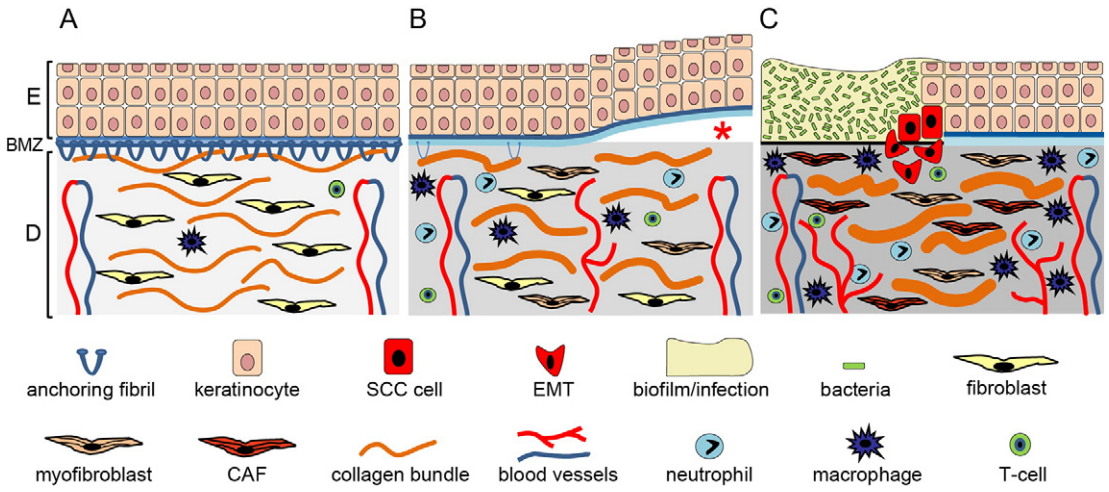

Fig. 1. Schematic of stromal modifications in recessive dystrophic epidermolysis bullosa (RDEB). (A) Normal skin. Type VII collagen (C7) is produced by keratinocytes and fibroblasts and is the main component of anchoring fibrils, which assure anchorage of the cutaneous basement membrane zone (BMZ) to the dermis. (B) RDEB blistering skin. C7 deficiency/absence in generalized RDEB affects anchoring fibril formation, thus leading to blisters (asterisk) beneath the BMZ. At sites of chronic blistering, inflammatory cells (neutrophils, macrophages and T-cells) increase in the dermis, which is being populated by myofibroblasts. The latter are responsible for enhanced fibrogenesis and release a high amount of growth factors, in particular TGF-β, and matrix components. (C) RDEB chronic wound. C7 deficiency affects the wound healing process by impairing keratinocyte and fibroblast migration, and also stimulates new vessel formation. At sites of unremitting wounds, continuous myofibroblast proliferation and matrix deposition lead to progressively increasing fibrosis characterized by augmented collagen bundle thickness and density, and matrix stiffness. In parallel, biofilm-producing bacteria colonize non-healing wounds and contribute to aggravate tissue inflammation. Altogether, these changes make the RDEB stroma a microenvironment prone to cancer initiation, where cells with features of cancer-associated fibroblasts (CAF-like cells) are found. Epithelial cells (keratinocytes) then acquire markers of epithelial-mesenchymal transition (EMT) and convert into carcinoma cells. In the tumor stroma, CAF contribute to squamous cell carcinoma (SCC) invasion and progression. E: epidermis, D: dermis, SCC: squamous cell carcinoma. Background darkening in (B) and (C) indicates increasing matrix stiffness.

SCC characterized so far have been shown to be not different from those found in usually less aggressive, UV-associated non-RDEB tumors [7–9]. Also the involvement of oncogenic papillomaviruses infection, postulated because of the presence of non-healing wounds, has been excluded as a cause of the aggressive nature of RDEB SCC [10]. Thus, alterations of the mesenchymal stroma rise as primary determinants for cancer development in RDEB [11].

Here, we review current evidence on the links between the C7 deficient stromal environment and the development of aggressive SCC in RDEB individuals. We further introduce highlights on potential targets for therapies aimed at counteracting the stroma pro-tumorigenic activity in RDEB skin.

Altered wound healing in RDEB

Physiological healing of wounded skin is a complex process in which sequential and partly overlapping events assure efficient restoration of tissue integrity. These events include blood coagulation, infiltration of the wound bed by inflammatory cells, re-epithelialization to recover skin barrier, formation of the granulation tissue, the wound transitory stroma, and tissue remodeling [12].

Two mouse models of RDEB, the C7 hypomorphic mouse, that expresses about 10% of C7 present in wild-type animals and shows phenotypic features comparable to severe RDEB [13], and a tamoxifen-inducible *Col7a1* knockout mouse that loses C7 after tamoxifen application but does not manifest chronic disease signs [3], have been used to investigate the wound healing process. These studies showed that C7 deficiency directly interferes with the healing process by altering the wound microenvironment [3] (Fig. 2). C7 loss results in impaired keratinocyte migration due to perturbed distribution of laminin-332 at the basement membrane zone (BMZ) and of its receptor integrin α6β4, associated with increased activation of laminin-332 signaling. Importantly, laminin-332 and integrin α6 disorganized expression is also found in wound biopsies from RDEB patients [3]. C7 loss also affects the migratory capacity of dermal fibroblasts, necessary for the release of the provisional wound matrix, and associates with increased transforming growth factor-β (TGF-β) signaling, resulting in delayed granulation tissue maturation and excessive collagen I release, and, overall, in delayed wound closure.