Research in practice: Towards deciphering the role of epidermal proteases in recessive dystrophic epidermolysis bullosa progression

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The clinical problem

Recessive dystrophic epidermolysis bullosa (RDEB) is an autosomal monogenic congenital skin blistering disorder. It manifests with unremitting blister formation leading to generalized painful chronic wounds of the skin and mucosa with multi-organ involvement. Chronically injured and inflamed skin leads to severe fibrosis, clinically evident by the development of mitten deformities [1]. Ultimately, inflammation and subsequent progressive fibrosis result in tissue stiffening, predisposing to the development of high-risk, early onset cutaneous squamous cell carcinomas (cSCCs), which are the main cause of patient death [2]. Cutaneous squamous cell carcinomas in RDEB patients are epidermally-driven tumors that develop mostly at the site of injury, suggesting a major role of the skin microenvironment in tumor initiation and development [3–5]. Recessive dystrophic epidermolysis bullosa is caused by mutations in the COL7A1 gene that encodes for the collagen VII protein. Collagen VII arranges in homotrimeric macromolecules in the extracellular matrix and forms anchoring fibrils at the skin basement membrane to ensure skin integrity. Absence of collagen VII results in poor cohesion between the skin epidermis and underlying dermis that is responsible for the extreme fragility of the skin to mechanical friction. Different therapies are currently developed to treat RDEB such as RNA-, gene- or cell-based therapies and symptom relief therapies using pharmaceutical compounds [6]. However, RDEB remains an incurable disease responsible for very poor quality of life and premature death [7]. A better understanding of the molecular mechanisms driving RDEB progression, including the role of the epidermis in cSCCs development, is a prerequisite for the development of innovative therapeutic strategies.

Research hypothesis

Our project is to assess the contribution of the epidermis and, in particular of epidermally secreted proteases, in driving RDEB disease progression and development of cSCCs. We focus on kallikreins (KLKs), a family of secreted proteases involved in inflammation and cancer initiation. We found that some KLKs are upregulated in our RDEB mouse model, as well as in patient skin and sera. Importantly, we have seen that the global proteolytic activity of the skin is also enhanced in patient samples (unpublished data), confirming very early studies of RDEB pathogenesis before the causative gene

Summary

Recessive dystrophic epidermolysis bullosa (RDEB) is an incurable severe skin disease caused by loss of collagen VII, an extracellular protein that ensures skin cohesion. It manifests in skin blistering and unresolved cycles of wounding and healing that progressively lead to dermal stiffening and early development of aggressive cutaneous squamous cell carcinomas. Inflammation and subsequent tissue fibrosis highly contribute to RDEB pathogenicity and targeting them could provide new therapeutic options. Kallikreins (KLKs) are epidermal secreted proteases, which contribute to skin desquamation and inflammation. Kallikreins are involved in the pathogenesis of several inflammatory skin disorders, but interestingly also in the initiation and progression of different cancers. Our project aims at deciphering the role of KLKs in inflammation, fibrosis, and tumor development in RDEB.
was identified [8]. We rationally hypothesize that KLKs act as pro-inflammatory and pro-tumorigenic molecules in the context of RDEB and participate to the disease progression and cancer initiation and/or development.

State of research

RDEB is an inflammation-driven skin disorder

A deep understanding of the mechanisms acting downstream collagen VII loss in RDEB is required for the development of innovative therapies for patients. With this aim our lab developed a RDEB mouse model that mimics the disease progression and recapitulates the main disease characteristics, such as progressive fusion of the digits in mouse forepaws [9]. Analysis of this mouse model revealed the decisive role of inflammation in RDEB pathology; these results were confirmed in patients [10–12]. Specifically, analysis of the RDEB mice back skin showed increased tissue inflammation with higher density of immune cells and elevated production of interleukin (IL)-6 and tumor necrosis factor (TNF)α, two pro-inflammatory cytokines [10]. Inflammation in RDEB is intrinsically linked with transforming growth factor beta (TGF)-β activity, a central player in several wound healing pathologies regulating both cell behavior, extracellular matrix production and the immune response [13]. As a consequence of unresolved cycles of wounding, healing and chronically injured skin, RDEB patients have enhanced production of TGF-β, thus creating a positive feedback loop promoting inflammation and fibrosis. Increased TGF-β activity was directly linked to disease severity in monozygotic twins with RDEB [11]. Additionally, inhibition of TGF-β either with the physiological decorin or with losartan ameliorates mitten deformities in RDEB mice by targeting inflammation and fibrosis [10, 14], while a first clinical trial on the use of losartan in RDEB is ongoing (EudraCT Number: 2015-003670-32). These studies illustrate the important role of TGF-β in RDEB.

Inflammation and subsequent tissue fibrosis progressively lead to dermal stiffening in RDEB patients, which predisposes to the development of high-risk early onset aggressive cSCCs. When compared to UV-induced SCCs, it appears that RDEB cSCCs are micro-environmentally driven and initiated by tissue damage, inflammation and dermal remodeling [12]. The dermal micromilieu undoubtedly plays a major role in the development of RDEB cSCCs, however RDEB SCCs show higher mutational rate than UV-induced SCCs and specific mutations, considering the patients’ age, suggesting that the epidermis itself can promote the aggressiveness of RDEB tumors [3, 12, 15]. Together with other biological processes, increased epithelial proliferation caused by constant wound healing attempts favors the acquisition of pro-tumorigenic mutations by keratinocytes [15]. Furthermore, as first barrier of the body, the epidermis secretes inflammatory cytokines to protect against external pathogens. Recessive dystrophic epidermolysis bullosa skin and associated SCCs are characterized by enhanced bacterial colonization, which consequently makes the proper regulation of skin inflammation challenging [12]. This suggests that the epidermis can itself be, at least in part, responsible for RDEB progression. As emerging regulators of skin inflammation and carcinogenesis, KLKs are good candidates for RDEB treatment.

KLKs contribute to the skin inflammation

Kallikreins, a family of 15 secreted serine proteases whose expression is restricted to the skin epidermis and appendages, contribute to the skin barrier function. They are secreted as pro-enzymes and get activated in the extracellular space by a cascade of proteolytic cleavages. Kallikreins activity is regulated by specific physiological inhibitors, e.g. the lympho-epithelial Kazal-type-related inhibitor (LEKTI) that is known to inhibit KLK5, KLK7 and KLK14, and skin pH [16]. Physiological roles of KLKs are skin desquamation, through degradation of corneodesmosomes and regulation of inflammation. Kallikreins participate to the skin barrier function by regulating the bioavailability of several proteins, such as the anti-microbial peptide LL-37 or filaggrin [17, 18]. Abnormal regulation of the KLK cascade is responsible for Netherton syndrome (NS) and contributes to atopic dermatitis (AD) and psoriasis pathogenesis, three skin inflammatory diseases. In the case of NS, loss of function of the SPINK5 gene encoding LEKTI, leads to over-activation of the KLK cascade. Upregulation of several pro-inflammatory cytokines is observed in NS including thymic stromal lymphopoietin (TSLP), which favors pro-inflammatory lymphocytes differentiation and ultimately impairment of the skin barrier function [19]. In AD, secretion of IL-4 and IL-13 cytokines by Th2 lymphocytes acts on KLK7 and its physiological inhibitor LEKTI and impacts degradation of corneodesmosomes, illustrating the many crosstalk interactions between inflammation and KLKs [20]. Similarly to RDEB, AD skin exhibits a higher bacterial colonization, which upregulates different KLKs including KLK6 [21]. These studies point to a direct link between KLKs and pro-inflammatory signaling. Few is known on the role of KLKs in psoriasis except that KLK6 and KLK7 are highly increased in psoriatic lesions [22] and that KLK8 is a marker of disease severity [22] and involved in microabscess formation [23]. Additional studies are required to decipher the precise involvement of KLKs in psoriatic lesions.

As mentioned above, TGF-β is a central player of RDEB promoting inflammation and fibrosis. Notably, multiple KLKs are able to process the latent form of TGF-β by
processing the LAP motif in seminal plasma [24]. Some evidence shows that KLKs are involved in wound healing, which is disturbed in RDEB. Kallikreins can cleave several extracellular matrix (ECM) proteins of the wound microenvironment, thus playing a role in all phases of the wound healing process, including remodeling of the wound [25]. Knockout of KLK8 in mice is associated with an upregulation of KLK6, resulting in impaired keratinocytes proliferation, differentiation and migration, as well as delayed wound closure [26]. Kallikrein-6 also directly impacts keratinocytes migration by regulating E-cadherin shedding, a protein required for cell-cell junctions [27]. The resulting impaired cell-cell adhesion enhances keratinocyte invasion capacity and favors initiation of cSCCs [27]. Another study showed that inhibition of KLK6 in mice reduces tumor development and decreases inflammation confirming its role in carcinogenesis [28]. Several other KLKs such as KLK5, KLK13, KLK7 and KLK4 were suggested as potential actors of cancer initiation and development [25]. In RDEB, cSCCs initiation is viewed as an outcome of pathological inflammation and unresolved wound healing and we rationally hypothesize that KLKs could contribute to this.

Potential involvement of KLKs in RDEB pathogenicity

Because of their preponderant role in skin inflammation, wound healing, and carcinogenesis, KLKs appeared as serious candidates for modulation of RDEB severity (Figure 1). Based on the evidence that KLK6 is a pro-inflammatory and pro-tumorigenic molecule, our project aims at deciphering the precise role of KLK6 and potentially other members of the KLK cascade in RDEB. We confirmed that KLK6 abundance and activity are modified both in our RDEB model and in patients during disease progression and associated with inflammation (unpublished data). We then used keratinocytes from healthy donors and RDEB patients to modulate KLK6 expression and showed that it modifies secretion of pro-inflammatory cytokines and regulation of TGF-β signaling. Interestingly, our experiments also revealed that KLK6 plays a role in fibrosis in RDEB, as it appears to regulate production and degradation of multiple pro-fibrotic extracellular matrix proteins (unpublished data). Our data reinforce the idea that although being produced by the epidermis, KLK6 actively contributes to the dermal extracellular matrix remodeling in the context of an injured microenvironment. These promising in vitro results encouraged us to generate KLK6 deficient RDEB animals to interrogate this hypothesis in an in vivo situation.

Conclusion from clinical practice

Our data propose KLK6 as a new potential target to limit inflammation and fibrosis in RDEB and highlight the contribution of the epidermis in RDEB progression. Our in vivo experiment will give us some clues on the role of KLK6 in

![Figure 1](image.jpg) Kallikreins as candidates promoting RDEB disease progression. Recessive dystrophic epidermolysis bullosa is caused by mutations in the COL7A1 gene, which lead to chronic skin fragility and ultimately to the development of early-onset aggressive cSCCs. We hypothesize that kallikreins act on inflammation and subsequent fibrosis, potentially through TGF-β, as well as on tumor initiation to promote RDEB progression (yellow arrows denote the tumor on the hand of an individual with severe generalized RDEB). Targeting kallikreins with specific inhibitors could prevent progression of RDEB.
tumor initiation in RDEB. While the development of specific KLKs inhibitors is challenging due to the high structural similarities in between the different KLKs, we are planning to use our RDEB mouse model in a pre-clinical study to validate our findings. Our study will pave the way for the development of new drugs that can specifically modulate KLKs activity and prevent progression of RDEB and possibly other fibrotic disorders.

Funding

This work was supported by EB Research Partnership, the German Research Foundation (DFG) through SFB1160 project B03 and by the Fritz Thyssen Foundation to DK.

Acknowledgements

Open access funding enabled and organized by Projekt DEAL.

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