Deletion of the epidermis derived laminin γ1 chain leads to defects in the regulation of late hair morphogenesis

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Abstract

Laminins are the most abundant non-collagenous basement membrane (BM) components, composed of an α, β and γ chain. The laminin γ1 chain, encoded by *LAMC1*, is the most abundant γ chain. The main laminin isoforms in the dermo-epidermal junction (DEJ) are laminin-332, laminin-511 and laminin-211, the latter being restricted to the lower part of hair follicles (HFs). Complete deletion of *LAMC1* results in lethality around embryonic day 5.5. To study the function of laminin γ1 containing isoforms in skin development and maturation after birth, we generated mice lacking *LAMC1* expression in basal keratinocytes (*LAMC1*^{EKO}) using the keratin 14 (K14) Cre/*loxP* system. This deletion resulted in loss of keratinocyte derived laminin-511 and in deposition of fibroblast derived laminin-211 throughout the whole DEJ. The DEJ in areas between hemidesmosomes was thickened, whereas hemidesmosome morphology was normal. Most strikingly, *LAMC1*^{EKO} mice showed delayed HF morphogenesis accompanied by reduced proliferation of hair matrix cells and impaired differentiation of hair shafts (HS). However, this deletion did not interfere with early HF development, since placode numbers and embryonic hair germ formation were not affected. Microarray analysis of skin revealed down regulation of mainly different hair keratins. This is due to reduced expression of transcription factors such as HoxC13, FoxN1, FoxQ1 and Msx2, known to regulate expression of hair keratins. While the role of laminin-511 in signaling during early hair germ formation and elongation phase has been described, we here demonstrate that epidermal laminin-511 is also a key regulator for later hair development and HS differentiation.

Introduction

BMs are highly structured extracellular matrices that form a dynamic interface separating and connecting simultaneously epidermal with mesenchymal compartments. Its components are regulators of cellular processes essential for skin integrity as illustrated by several acquired and inherited diseases

[1,2]. The major components of the DEJ are members of the laminin, nidogen, collagen IV and the heparan sulfate proteoglycan (perlecan and agrin families) [3,4].

Laminins are the most abundant non-collagenous structural components of BMs and play important roles in tissue morphogenesis and homeostasis by regulating cell survival, differentiation, migration and adhesion [5,6]. Furthermore, they link the BM to epithelial transmembrane receptors, primarily β1 and β4 integrins [7]. Laminins are heterotrimeric proteins composed of α, β, and γ subunits [8]. Currently, 5 α-, 3 β- and 3 γ-subunits have been identified in mice and humans forming the 18 known laminin heterotrimers [5,9]. The different isoforms exhibit tissue and developmental specificity [10–12], reflecting their diverse functions in vivo.

Laminins in the skin have been implicated in the regulation of HF development, driven by a series of epithelial-mesenchymal crosstalk [13]. HF development is initiated by the induction of epithelial hair placodes by the first message of the underlying mesenchyme [14] and proceeds with the down growth of hair germs into the dermis to form HFs [15]. Induced by the epithelial message derived from elongating HFs, dermal papilla cells condensate and become surrounded by the hair bulb. Signals from dermal papilla cells stimulate proliferation of adjacent hair matrix cells of the HF. During HF differentiation, matrix cells produce progenitors that differentiate and give rise to HS and inner root sheath of the HFs [16–18,13]. As the cells move distally into the different HF compartments, they start to differentiate, visible by the expression of characteristic marker proteins (keratins). Differentiation of matrix cells is regulated by a number of factors, such as Wnt/Lymphoid enhancing factor 1 (Lef1) [19,20], fibroblast growth factor 7 (Fgf7) [21] and members of the bone morphogenetic protein (BMP) family [22,23]. These are important in HF proliferation and differentiation during hair morphogenesis. However, the role of extracellular matrix proteins, which provide critical anchorage to the cells, in this process is not elucidated.

The laminin γ1 chain is the most ubiquitously expressed γ subunit, present in 10 of the 18 known laminin isoforms [5]. Previous studies showed that laminin γ1 is a prerequisite for the formation of an embryonic BM, since *LAMC1* complete knock out mice die at embryonic day (E 5.5) [24]. Expression of laminin-111 and -511 is already detected at the morula stage in mouse development [25]. At the DEJ, laminins are synthesized by both dermal [26,27] and epidermal cells [28]. The main secreted laminin variants at the DEJ are laminin-332 and laminin-511. Depending on the developmental stage, laminin-211 is also present in the lower part of the HFs [29,9]. During HF elongation, the laminin composition of the BM undergoes changes: while laminin-511 expression pattern is not altered, laminin-332 [30] and laminin-111 [31] are downregulated. Previous studies have shown the importance of laminin-511 in early embryonic hair germ elongation. The loss of laminin-511 in transgenic mice resulted in a reduced follicular proliferation, thus in a reduced number of hair germs after regression of the HFs [25]. Further known laminin γ1 chain containing isoforms in the skin are laminin-311, and -321, however, their presence in murine skin is discussed controversially [9].

To study the function of laminin γ1 chain containing isoforms in skin development and maturation in later stages, and to avoid early embryonic lethality, we generated mice that do not express *LAMC1* in basal keratinocytes from early developmental stages onwards (referred to as $LAMC1^{EKO}$) using the K14 Cre/*loxP* system [32].

This report demonstrates that epidermal derived laminin γ1 chain is a key regulator for late hair morphogenesis and differentiation. We showed that the deletion of epidermal laminin γ1 resulted in the loss of keratinocyte derived laminin-511 and in ectopic deposition of fibroblast derived laminin-211 in the whole DEJ. These changes in laminin composition led to ultrastructural defects of the dermo-epidermal BM zone between hemidesmosomes and in delayed hair growth, accompanied by reduced proliferation of hair matrix cells and defects in hair differentiation. Furthermore, our studies suggest an important role of the *BMP-MSX2-HOXC13-FOXN1* signaling axis in regulation of the processes required for HF differentiation and proliferation.

Results

Depletion of keratinocyte specific laminin γ1 from the dermo-epidermal BM

The main laminins in the murine dermo-epidermal BM are laminin-332 and the laminin γ1 chain containing isoform laminin-511 and, in the lower part of the HF, laminin-211.

To analyze the functional impact of the γ1 chain containing laminins in late hair development, homozygous floxed laminin γ1 mice [33] were crossed with a transgenic mouse strain expressing Cre-recombinase under the control of the human K14 promoter [32]. This results in the mouse strain $LAMC1^{EKO}$ which lacks laminin γ1 chain expression and secretion by basal keratinocytes. The human K14 promoter becomes active at around E14.5, and activity in postnatal mice is restricted to the mitotically active basal keratinocytes, the outer root sheath of the HFs and the oral epithelium [34]. After birth, homozygous $LAMC1^{EKO}$ mice were