Keratin Isotypes Control Desmosome Stability and Dynamics through PKCα

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Expression and interaction of desmosomal components and keratins provide stable cell cohesion and protect the epidermis against various types of stress. The differentiation-specific isotype composition of the keratin cytoskeleton and desmosomes is regarded as a major determinant of adhesive strength. In support, wound healing is characterized by a transient decrease in desmosomal adhesion accompanied by increased expression of keratins K6/K16/K17 at the expense of K1/K10. The significance of altered keratin expression for desmosomal composition and adhesion remains incompletely understood at a mechanistic and functional level. Here, we investigated the respective contribution of K5/K14 or K6/K17 to desmosome adhesion, on their stable re-expression in keratinocytes lacking all keratins. This revealed that K5/K14 filaments support stable desmosomes, whereas "wound healing" keratins K6/K17 induce elevated protein kinase C alpha–mediated desmosome disassembly and subsequent destabilization of epithelial sheets. Moreover, our data suggest that K5/K14 sequester protein kinase C alpha in the cytoplasm, whereas K6/K17 or the absence of all keratins enables protein kinase C alpha translocation to the plasma membrane and induction of desmosome disassembly. Gain- and loss-of-function experiments support a major role of K5 in desmosome stability control via protein kinase C alpha. Our data show that keratin isotypes differently and specifically regulate wound healing and invasion by modulating intercellular adhesion.

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INTRODUCTION

The epidermis has evolved to protect the body against mechanical stress, infections, and dehydration by virtue of strong intercellular adhesion among keratinocytes. Stable intercellular adhesion and force resilience are controlled by several types of cell adhesion complexes attached to the cytoskeleton. Among these, the keratin-desmosome scaffold has a dual role in regulating epidermal differentiation through crosstalk with growth factors, in addition to mediating intercellular adhesion (Homberg and Magin, 2014; Loschke et al., 2015; Simpson et al., 2011). To adapt keratinocyte adhesion and function to requirements of differentiation, wound healing, and pathogenesis, the keratin-desmosome complex requires remodeling at the level of protein composition, interactions, and posttranslational modifications (PTMs) (Albrecht et al., 2015; Simpson et al., 2011). Keratins (K) are encoded by a large gene family of 28 type I and 26 type II keratins expressed in a pairwise fashion to ensure the formation of the intermediate filament cytoskeleton in all epithelia from heterodimeric subunits in a cell-specific and differentiation-dependent manner (Hesse et al., 2004; Magin et al., 2007). Ultimately, keratin intermediate filaments are organized as networks that attach to desmosomes and hemidesmosomes, thereby contributing to the mechanical and signaling properties of epithelia (Pan et al., 2013; Windoffer et al., 2011). The basal, proliferative compartment of the epidermis predominantly expresses the keratin pair K5/K14, which on terminal differentiation is replaced by K1/K10. This default pattern is altered during barrier disruption, wounding, tissue regeneration, and malignant transformation, conditions that require transient decreased intercellular adhesion, enhanced proliferation, and migration of keratinocytes. In these settings, K6, K16, and K17 are rapidly and transiently expressed at the expense of K1/K10. Although in vitro data show that K5/K14 protein complexes are much more stable than those between K6/K16/K17 (Hatzfeld and Franke, 1985), the contribution of keratin isotypes to desmosome-mediated keratinocyte adhesion during differentiation and wound healing is not fully resolved. During epidermal injury, re-epithelialization is the most crucial process, as its failure underlies chronic, nonhealing wounds, a clinically highly relevant problem (Gurtner et al., 2008). Re-epithelialization involves altered adhesion, migration, and proliferation of keratinocytes at the wound edge to enable wound closure and restoration of the epidermal barrier (Shaw and Martin, 2009). Among the earliest changes detectable in keratinocytes at the wound margin are diminished contacts of keratins to desmosomal proteins and loss of desmosome hyperadhesion (Garrod et al., 2005; Paladini et al., 1996), followed by increased keratinocyte migration mediated by a K6-Src sequestration mechanism (Rotty and Coulombe, 2012). Delayed wound healing in protein kinase C alpha ($PKC\alpha^{-/-}$) mice supports a major contribution of PKCα in

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Abbreviations: DP, desmoplakin; Dsg, desmoglein; K, keratin; KIF, keratin intermediate filament; KtyI, keratin type I gene cluster; KtyII, keratin type II gene cluster; PKCα, protein kinase C alpha; PKP, plakophilin; PM, plasma membrane; WB, Western blotting; WT, wild-type

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regulating desmosome adhesion. Conversely, overexpression of PKCα accelerated wound healing by decreasing desmosome adhesion (Thomason et al., 2012). Yet, these studies have not examined expression and contribution of keratin isotypes to PKCα regulation.

Altogether, these findings raise the question to which extent the composition and abundance of keratin intermediate filaments contribute to desmosome adhesion and epithelial stability. Previously, we reported that the absence of all keratins in keratinocytes rendered epithelial sheets fragile on exposure to mechanical stress, but that moderate expression levels of K5/K14 rescued sheet integrity (Kroger et al., 2013). Also, our data indicated that keratins sequester PKCα through the scaffold protein Rack1, thereby limiting phosphorylation of desmosomal proteins necessary for desmosome stability (Kroger et al., 2013). To further dissect isotype-specific keratin functions, we established stable keratinocyte cell lines re-expressing type I keratins K14 or K17 or type II keratins K5 or K6 in the corresponding keratin null background. This revealed that cells expressing K6 or K17 show elevated, PKCα-mediated desmosome disassembly and subsequent destabilization of epithelial sheets. In contrast, keratinocytes expressing K5 or K14 displayed stable desmosomes, suggesting that expression of "wound healing" keratins weakens intercellular adhesion. Furthermore, gain- and loss-of-function studies suggest that the type II keratin K5 is a major determinant of desmosome stability, whereas type I keratins seem to play a minor role.

RESULTS AND DISCUSSION

Expression of K14, but not of K17, stabilizes desmosomes

Recently, lack of keratins in the epidermis of mice was found to diminish the size and number of desmosomes along the plasma membrane (PM), accompanied by accumulation of desmosomal proteins in the cytoplasm (Bar et al., 2014; Kroger et al., 2013). Mechanistic studies using cultured keratinocytes showed that under steady-state conditions, keratins restrict desmosomal protein phosphorylation in a PKCα-dependent manner and thereby stabilize desmosomes at the PM (Kroger et al., 2013). To further examine the contribution of different keratin isoforms to epithelial integrity, maintenance, and dynamics of junctions, we established different cell lines expressing only distinct keratin isotypes. By lentiviral transduction of cells lacking the entire keratin type I gene cluster (KtyI$^{-/-}$), we generated cells expressing either K14 (KtyI$^{-/-}$K14; K14 cells) or K17 (KtyI$^{-/-}$K17; K17 cells) as the sole type I keratin. In the background of cells lacking the entire keratin type II gene cluster (KtyII$^{-/-}$), we expressed K5 (KtyII$^{-/-}$K5; K5 cells) or K6 (KtyII$^{-/-}$K6; K6 cells) as the sole type II keratin.

Genetically, KtyI$^{-/-}$ cells lack all type I keratin genes but maintain type II keratin gene expression (Ramms et al., 2013). At the mRNA level, no type I keratin is detectable, as shown for K14 and K17, whereas type II keratin genes were transcribed, as shown for K5 and K6 (Supplementary Figure S1a online). Western blotting (WB) revealed the absence of type I and the strong reduction of type II keratins at the protein level (Figure 1a). Immunostainings confirmed the absence of keratin filaments in KtyI$^{-/-}$ compared with wild-type (WT) cells (Figure 1b–c‴). In KtyI$^{-/-}$ cells, after stable transduction with K14 or K17 cDNAs, also K5 and K6 are present at the protein level, next to K14 or K17 (Figure 1a–a′). WB of total protein lysates showed similar levels of K14 (38%) and K17 (31%), relative to their expression in WT cells (Figure 1a′). The level of K5 protein is higher in K14 compared with K17 cells, the amount of K6 is higher in K17 compared with K14 cells (Figure 1a′). At the same time, the overall amount of unsoluble keratin proteins was slightly diminished in K17 cells compared with K14 cells, as documented by Coomassie Blue staining of the IF-enriched protein fractions separated by SDS-PAGE (Supplementary Figure S1b–b′). The unaltered mRNA levels of these type II keratins in both K14 and K17 cells point toward a posttranslational regulatory mechanism (Supplementary Figure S1a). Analysis of protein half-life times, after cell treatment with cycloheximide and subsequent WB, revealed increased stability of K5 and accelerated degradation of K6 in the presence of K14 and increased stability of K6 along with increased degradation of K5 in the presence of K17 (Supplementary Figure S1c–c′). In support, the stability of heteromeric complexes between distinct keratins is different in vitro, although all type I keratins can pair with all type II keratins (Hatzfeld and Franke, 1985). Our data indicate that specific keratin pairs, such as K5/K14 and K6/K17, are formed preferentially in vivo. Furthermore, quantitative PCR and WB suggest that selectivity of certain keratin pairs occurs at the protein level and is not due to differences at the transcript level (Figure 1a–a′, Supplementary Figure S1a–c′). Whether this difference in stability is related to differences in affinities or is affected by different PTMs of keratins remains to be shown. Irrespective of composition, confocal microscopy of K5, K14, K6, and K17 showed WT-like cytoskeletal organization in both cell lines (Figure 1d–e‴).

To investigate whether intercellular adhesive strength depends on distinct keratin isotypes, a mechanical cell dissociation assay was performed. In this assay, confluent cell monolayers were lifted from the cell culture dish by dispase treatment and subjected to mechanical stress (Calautti et al., 1998). Loss of all keratins weakens intercellular adhesive strength to a significant extent, as shown by a greater than 100-fold increase in the number of sheet fragments, compared with WT controls (Homberg et al., 2015; Kroger et al., 2013) (Figure 2a, a′, a‴). Presently, we cannot exclude a partial contribution from cytolyzing, in addition to disturbed desmosomes, to the impaired epithelial sheet integrity in the mechanical dispase assay. The expression of K14 restored epithelial sheet integrity to a significant extent, in contrast to K17 cells (Figure 2a″–a‴). Confocal microscopy revealed a considerable decrease of DP1/2 at the PM and accumulation of DP1/2 in the cytoplasm of KtyI$^{-/-}$ and K17 cells, compared with WT and K14 cells (Figure 2b–b‴). Desmoglein (Dsg) and desmoplakin (DP) colocalize to some extent in the cytoplasm of K17 cells (Supplementary Figure S2a online), indicating that some desmosomal proteins are internalized together (McHarg et al., 2014). Similar to KtyI$^{-/-}$ and KtyII$^{-/-}$ cells (Homberg et al., 2015; Kroger et al., 2013) (Supplementary Figure S2c), the level of desmosomal proteins in K17 cells is strongly decreased, ranging from 76% for Dsg1/2, 40% for plakophilin 1, 45% for plakophilin 3, to 88% for DP1/2 after 72 hours, compared