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Constitutive Autophagy and Nucleophagy during Epidermal Differentiation

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Epidermal keratinocytes migrate through the epidermis up to the granular layer where, on terminal differentiation, they progressively lose organelles and convert into anucleate cells or corneocytes. Our report explores the role of autophagy in ensuring epidermal function providing the first comprehensive profile of autophagy marker expression in developing epidermis. We show that autophagy is constitutively active in the epidermal granular layer where by electron microscopy we identified double-membrane autophagosomes. We demonstrate that differentiating keratinocytes undergo a selective form of nucleophagy characterized by accumulation of microtubule-associated protein light chain 3/lysosomal-associated membrane protein 2/p62 positive autolysosomes. These perinuclear vesicles displayed positivity for histone interacting protein, heterochromatin protein 1α, and localize in proximity with Lamin A and B1 accumulation, whereas in newborn mice and adult human skin, we report LC3 puncta coincident with misshaped nuclei within the granular layer. This process relies on autophagy integrity as confirmed by lack of nucleophagy in differentiating keratinocytes depleted from WD repeat domain phosphoinositide interacting 1 or Unc-51 like autophagy activating kinase 1. Final validation into a skin disease model showed that impaired autophagy contributes to the pathogenesis of psoriasis. Lack of LC3 expression in psoriatic skin lesions correlates with parakeratosis and deregulated expression or location of most of the autophagic markers. Our findings may have implications and improve treatment options for patients with epidermal barrier defects.

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INTRODUCTION

The epidermis is a multilayered structure continuously renewed by keratinocytes of the basal layer that divide and differentiate to form cells of the spinous, granular, and cornified layers. The proliferating basal layer is a heterogeneous population comprising epidermal stem cells and transit-amplifying cells that have limited self-renewal capacity and undergo differentiation after a few cycles (Watt, 1998). In the granular layer, keratinocytes begin to lose their organelles, and express structural protein characteristic for epidermal terminal differentiation, leading to the flattening, collapse, and the eventual death of the cells. These flattened cells, corneocytes, which form the cornified layer, are rich in proteins and are embedded in a lipid matrix, giving the epidermis its water-retaining, chemical, and mechanical properties, ensuring effective epidermal barrier function (Blank, 1953; Candi et al., 2005; Proksch et al., 2008).

Autophagy is generally used to describe cellular processes leading to the degradation of cytoplasmic components within lysosomes (Klionsky et al., 2016; Levine and Klionsky, 2004; Mizushima, 2007; Shintani and Klionsky, 2004). Macroautophagy (commonly referred to as autophagy) is a conserved catabolic process characterized by formation of intracellular double-membrane structures that degrade and recycle cytosolic proteins and organelles (Arstila and Trump, 1968; Mizushima, 2007). Specialized forms of autophagy are directed at specific organelles such as mitophagy (mitochondria), nucleophagy (nuclei) (Cecconi and Levine, 2008; Klionsky et al., 2007; Levine and Kroemer, 2008; McGee et al., 2011; Mizushima and Levine, 2010; Park et al., 2009), and more recently "ER-phagy" (endoplasmic reticulum) (Khaminets et al., 2015; Mochida et al., 2015). Autophagy in keratinocytes so far has been described as a mechanism of senescent cell death (Deruy et al., 2010, Gosselin et al., 2009), stress response leading to expression of early markers of differentiation and eventual cell death (Aymard et al., 2011), as well as a prosurvival mechanism that protects from UV-induced damage (Qiang et al., 2013; Yang et al., 2012; Zhao et al., 2013). However, most published work has been performed in monolayer keratinocyte culture that may not fully represent the stratified in vivo situation. A key regulator of epidermal development and

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Abbreviations: AKT, acutely transforming retrovirus AKT8 in rodent T-cell lymphoma; HP1α, heterochromatin protein 1α; LMNA, lamin A; LMNB1, lamin B1

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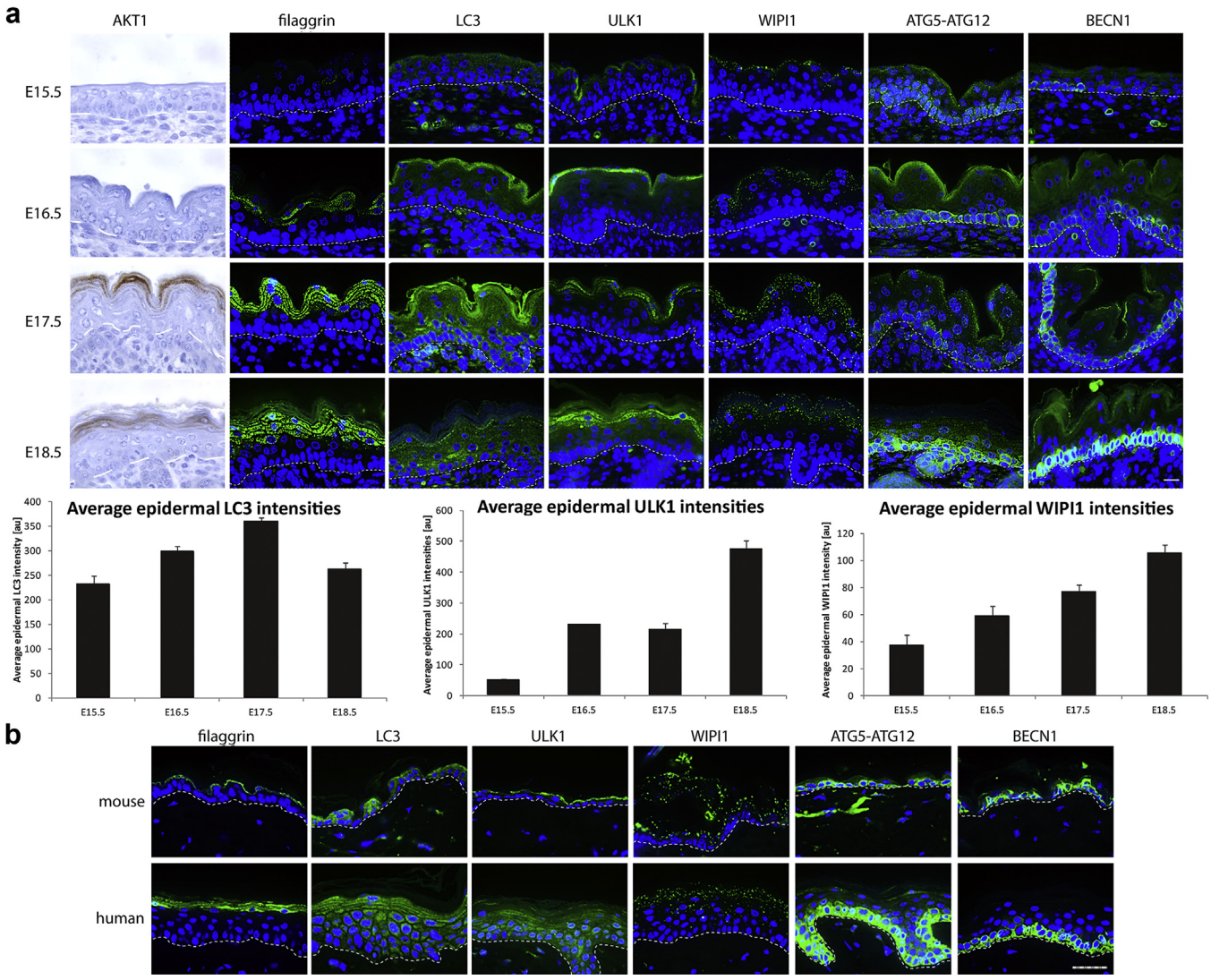

Figure 1. Induction of epidermal terminal differentiation during fetal development is accompanied by activation of autophagy marker expression. (**a**) Expression of acutely transforming retrovirus AKT8 in rodent T-cell lymphoma 1 (AKT1), epidermal terminal differentiation marker, filaggrin, and autophagy proteins LC3, ULK1, WIPI1, BECN1, and ATG5-ATG12 in mouse fetal skin development. Epidermal expression levels of LC3, ULK1, and WIPI1 were quantified in n = 3 fetal mouse samples from E15.5 to E18.5. ANOVA for average intensities from n = 3 samples of epidermal LC3 ($P < 0.00005$), ULK1 ($P < 0.0005$), WIPI1 ($p < 0.005$), respectively. (**b**). Expression of epidermal terminal differentiation and autophagy markers in both adult mouse and adult human epidermis. Bar = 20 μm. Dotted line = basement membrane. ANOVA, analysis of variance; ATG5-ATG12, autophagy related 5-autophagy related 12; BECN1, beclin 1; LC3, microtubule-associated protein light chain 3; ULK1, Unc-51 like autophagy activating kinase 1; WIPI1, WD repeat domain phosphoinositide interacting 1.

differentiation is the acutely transforming retrovirus AKT8 in rodent T-cell lymphoma (AKT)/mechanistic target of rapamycin pathway. Downstream of AKT is mTORC1 that regulates anabolic and catabolic processes including autophagy. Hyperactivation of mTORC1 signaling has been associated with a defective epidermal barrier in psoriasis (Buerger et al., 2013; Kjellerup et al., 2009) and vitamin D analogs used to treat epidermal barrier defect diseases such as psoriasis induce autophagy in cultured cells (Wang and Levine, 2011). Here we investigate the role of epidermal autophagy and its link with epidermal terminal differentiation.

RESULTS

Autophagy is involved in epidermal granular layer formation

During fetal development, expression studies are possible because of the temporal separation of epidermal terminal differentiation and skin barrier formation. We examined expression patterns of ULK1, beclin 1, WIPI1, autophagy related 5-autophagy related 12 complex, and LC3 as key markers of sequential stages of autophagy in the mouse embryo (Figure 1a). Epidermal barrier formation correlates with development of the cornified layer that occurs between E15.5 and E18.5 in mice. In E16.5 mouse fetal skin, filaggrin expression confirmed activation of terminal differentiation and granular layer formation. Filaggrin is also constitutively expressed in the granular layer of adult human skin (Figure 1b and Supplementary Figure S1b online), and is further increased in fetal granular layers at E17.5 and E18.5, when AKT1 is also expressed, indicating the presence of an intact granular layer. At E15.5, before granular layer development, the epidermis consists of proliferating basal and spinous layers (Supplementary Figure S1a). At this time point, LC3 as