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Chapter 5

Keratinocytes: Gatekeeper in Innate Defense

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Abstract

Keratinocytes, as a major cell type of skin epidermis, function as a gatekeeper to effectively prevent pathogen entry or long-term survival on the skin. Multiple pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) are expressed on keratinocytes, which enables keratinocyte recognition of microbial invasion. The activation of PRRs in keratinocytes in turn triggers the release of soluble effectors, such as the antimicrobial peptides that rapidly repel microbial assault. This short communication focuses on describing the expression profile of PRRs in keratinocytes and antimicrobial intermediates produced by keratinocytes after PRR activation, and highlights protective roles of keratinocytes in innate defense.

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The skin is the primary interface between the host and environmental damage such as microbes. A myriad of microbes colonize on skin and make contact with keratinocytes in the epidermis.

However, despite the abundant colonization by microbes, skin normally isn't infected or inflamed. Therefore, keratinocytes, the predominant cell type in the epidermis of skin, not only play an important role in maintaining the physical barrier between the host and the environment, but also participate in cutaneous immune responses to protect skin from infection (Kupper and Fuhlbrigge, 2004; Robert and Kupper, 1999). This short communication will describe how Toll-like receptors are activated to release antimicrobial peptides against pathogens in keratinocytes, thus maintaining skin homeostasis.

Pattern-Recognition Receptors in Keratinocytes

Toll-like receptors (TLRs) are a member of the pattern-recognition receptor (PRR) family and recognize structurally conserved molecules derived from microbes that are named pathogen-associated molecular patterns (PAPMs). As a consequence of its location, keratinocytes are exposed to millions of microbes and are endowed with an array of Toll-like receptors. It has been shown that TLRs1-10 are expressed in keratinocytes (Baker et al., 2003; Kollisch et al., 2005; Lebre et al., 2007; Schauber et al., 2007). Among these TLRs, TLR2, TLR3, TLR5 are constitutively expressed in primary human keratinocytes and immortalized human keratinocyte cell line HaCat while TLR4 was only found in HaCat cells (Kollisch et al., 2005).

However, some controversial results show no surface expression of TLR2, TLR4, TLR9 in cultured keratinocytes (Curry et al., 2003). One explanation is that the expression and function of TLRs are age-dependent.

The work done by the Elbe-Burge group has shown that significantly higher mRNA expression levels of TLRs 1-5 and the TLR4 co-factor MD2 were observed in embryonic and fetal skin (specific in keratinocytes) when compared with adult skin. TLR6 was essentially equally expressed in embryonic and fetal skin while TLR7-9 were absent in adult skin, but weekly detectable in prenatal skin(Iram et al., 2012). In addition to the expression of TLRs, the function of TLRs has age-dependent changes. The best evidence is exemplified by the fact that TLR3 exhibits marked differences in the magnitude of expression and function in keratincoytes before and after birth when compared with adults. Compared to basal keratinocytes from adults,

neonatal keratinocytes secrete high levels of CXCL8, CXCL10 and TNFα upon stimulation by TLR3 ligand poly(I:C) even though keratinocytes from both age groups express comparable levels of TLR3. Moreover, fetal skin keratinocytes were already able to respond to poly(I:C) with a similar strength to neonatal keratinocytes(Iram et al., 2012). The data suggests the existence of age-specific responses, rather than a global, linear progression from a prenatal to an adult pattern. However, the mechanisms underlying these differences remain unknown.

Besides TLRs, keratinocytes express other PRRs including nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) and C-type lectin receptors (CLRs). NOD2 expression was increased by peptidoglycan (PGN) and one of CLRs dectin-1 was enhanced by its ligand beta-glucan in keratinocytes (Kobayashi et al., 2009). Moreover, our unpublished data show that poly(I:C) increases both TLR3 and RIG-I expression. Toll-like receptors recognize pathogens at the cell surface (TLRs 1,2,4-6, 10) or within the endosome (TLRs 3, 7-9), whereas NLRs and RLRs act as intracellular surveillance molecules(Kumar et al., 2009). NLRs not only function in pathogen recognition but also play a role in tissue homeostasis(Kufer and Sansonetti, 2011). RLRs are crucial for host antiviral defense and sense double-stranded (ds) RNA. Viral dsRNA or synthetic ds RNA poly(I:C) is recognized by TLR3 and MDA5. The activation of these PRRs by PAMPs or endogenous signals of injury enables keratinocytes rapidly produce cytokines, antimicrobial peptides or antimicrobial intermediates (e.g. radical oxygen species and nitric oxide) in response to those damages.

Antimicrobial Intermediates in Keratinocytes

Cytokines, chemokines and antimicrobial peptides/proteins (AMPs) are major antimicrobial intermediates secreted by keratinocytes upon exposure to microbes. Upon stimulation by microbial pathogens, activation of TLRs leads to the production of interferons and inflammatory cytokines. These cytokines then activate surrounding cells to produce chemokines to recruit various inflammatory cells, such as neutrophils and macrophages, into the infected sites, thus clearing invading pathogens.

Except for cytokines and chemokines, the endogenous antimicrobial peptides secreted by keratinocytes are effector molecules of the innate host defense system (Ganz, 1999; Zasloff, 2002). Upon infection and injury, keratinocytes are able to produce AMPs within a few minutes. Around 10 antimicrobial peptides and proteins have been identified in keratinocytes, including cathelicidins (Braff et al., 2005), beta-defensins (Liu et al., 2002), regenerating islet-derived proteins (Lai et al., 2012) and others. These AMPs have a broad antimicrobial spectrum and inactivate microorganisms by direct interaction with biomembranes or other organelles. Besides their direct antimicrobial function, it has been suggested that AMPs play multiple roles as mediators of inflammation with impact on epithelial and inflammatory cells, influencing diverse processes such as cytokine release, cell proliferation, angiogenesis, wound healing, chemotaxis, immune induction, and protease antiprotease balance(Brown and Hancock, 2006; Lai et al., 2012; Yang et al., 2004).

PRRs Regulate Antimicrobial Intermediates in Keratinocytes

Human skin is exposed to millions of microbial organisms, and these microorganisms produce various kinds of PRR ligands. Keratinocytes are microbe-infected target cells and are equipped with a broad antimicrobial defense program enabling them to efficiently protect the host from microbial infection.

This protective function is partly mediated by the presence of AMPs as well as inflammatory cytokines and chemokines after PRRs have been activated in keratinocytes.

For example, peptidoglycan and lipoteichoic acid from *S. aureus* activated TLR2 and NOD2 on keratinocytes, resulting in activation of NF-κB and subsequent production of the neutrophil chemotactic factor IL-8 and iNOS (Mempel et al., 2003; Muller-Anstett et al., 2010), thus inhibiting *S.aureus* from invading. Other studies have shown that upon TLR3 ligation, fetal keratinocytes were able to produce CXCL9-11 and CCL3-5 that play a potential role in host defense against HSV-1(Iram et al., 2012; Nakayama et al., 2006). In addition to CXCL9-11 and CCL3-5, activation of TLR3 in cultured human keratinocytes induced production of TNFα, IL-18, and type I interferon (IFNα/β) and the development of Th-1 type immune responses

(Lebre et al., 2003). Moreover, our data showed that RNA from necrotic cells triggered TLR3 on undamaged keratinocytes around wounds to produce pro-inflammatory cytokines IL-6 and TNFα after a skin injury (Lai et al., 2009), thus helping to keep wounds sterile. Altogether, these data suggest that keratinocytes, via TLR activation, play an important protective role in microbial infections and skin injury.

In addition to inflammatory cytokines and chemokines, our recent data shows that expression of murine beta-defensin was upregulated by bacterial lipopeptides in wild-type keratinocytes, while it was attenuated in TLR2-deficient keratinocytes in vitro (Lai et al., 2010). In line with decreased the expression of beta-defensins, the infection of *S.aureus* in TLR2-deficient mice was increased (Lai's unpublished data). Moreover, recent work showed hormonally active vitamin D(3)-1,25- dihydroxyvitamin D(3) (1,25D3) acted as a signaling molecule in cutaneous immunity by increasing pattern recognition through Toll-like receptor-2 (TLR2), increasing the expression and function of the antimicrobial peptide cathelicidin, and killing off intracellular Mycobacterium tuberculosis (Liu et al., 2006). In addition, the Gallo group found that the epigenetic control of gene transcription by histone acetylation is important for 1,25D3-regulated antimicrobial and TLR function of keratinocytes against *S.aureus* (Schauber et al., 2008). Beside infection, skin injury also enhanced TLR2 function to induced antimicrobial peptide expression (Schauber et al., 2007), thus promoting wound healing (Lai et al., 2012).

Conclusion

Keratinocytes, the major constituents of the epidermis, produce cytokines and antimicrobial peptides/proteins via activation of PRRs right after skin is exposed to environmental damages. Keratinocytes provide a quick innate immune response to protect the host, functioning as a gatekeeper. Furthermore, the activation of PRRs in keratinocytes plays a vital role in skin infection and injury, thereby making them potential therapeutic targets. Therefore, the ability of PRRs to combat those diseases could be used in a dermatological clinic through the development of drugs that act as PRRs agonists or antagonists.

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