Epidermal activation of the small GTPase Rac1 in psoriasis pathogenesis

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ABSTRACT

The small GTPase Ras-related C3 botulinum toxin substrate 1 (RAC1) plays a central role in skin homeostasis, including barrier function, wound healing and inflammatory responses. Psoriasis is a common skin disease characterized by deregulation of these functions, and affected skin exhibit keratinocyte hyperproliferation, inflammation and immune cell infiltration. Although psoriasis is often triggered by environmental stimulus, there is a strong genetic association with genes expressed in both immune cells and keratinocytes, of which several are linked to Rac1 signaling. Rac1 is highly active in human psoriatic lesional skin and keratinocytes, and keratinocyte-specific overexpression of an activated mutant of Rac1, Rac1V12, in a transgenic mouse model closely mimics the presentation of human psoriasis. Both Rac1 activation in keratinocytes and immune derived stimulus are required to drive psoriasiform signaling in transgenic mouse and human xenograft models of psoriasis. Therefore, understanding how increased Rac1 activation in psoriatic epidermis is regulated is central to understanding how the abnormal crosstalk between keratinocytes and immune cells is maintained.

KEYWORDS

crosstalk; epidermis-immune; keratinocyte; psoriasis; Rac1

The small GTPase Ras-related C3 botulinum toxin substrate 1 (RAC1) plays an important role in epidermal homeostasis. Rac1 regulates epithelial junctions central for skin barrier integrity, coordinated collective migration of keratinocytes and stem cell maintenance. Further, Rac1 activity determines downstream signaling of many growth factors and cytokines, responsiveness to environmental extracellular stimulus and propagating inflammatory responses. These intracellular signal transduction pathways include STAT3-phosphorylation, binding and nuclear translocation, as well as NFκB signaling and promotes keratinocyte immune-cell crosstalk. One example of increased epidermal Rac1 activity is during wound healing, where Rac1 is essential for wound re-epithelialization. Another is through CD44 receptor binding on epithelial cells, which is the receptor for hyaluronic acid. CD44 receptor mediated Rac1 activation can also be induced by molecular mimicry of the group A Streptococcus (GAS) hyaluronic acid capsular polysaccharide. This triggers cytoskeletal rearrangements and result in increased permeability of intercellular junctions.

A common skin disease associated with abnormal wound healing responses (known as the Koebner phenomena), GAS infection and epidermal STAT3 activation is psoriasis, affecting roughly 1–3% of the population, and genetically associated with genes expressed in both keratinocytes and immune cells. Psoriasis commonly presents as erythematous scaly plaques on affected skin, and is associated with systemic co-morbidities such as psoriatic arthritis in up to 30% of cases. Interestingly, thioguanine, a Rac1 inhibitor has demonstrated efficacy in psoriasis, and Rac1 inhibitory peptides have shown promise in reducing antibody production and paw swelling in a murine collagen-induced arthritis model of rheumatoid arthritis. Therefore, we elucidated whether abnormal Rac1 activity was a feature of psoriatic lesional skin.

We found a marked activation of Rac1 in psoriatic lesional epidermis compared with normal control skin in a panel of lesional psoriasis patient skin and normal control skin. Further, increased Rac1 activation was also evident in non-lesional psoriasis skin biopsies. Isolating psoriasis patient keratinocytes and stimulating them with growth factors and cytokines such as EGF, TNFa, IL17 or IL22 induced strong Rac1 activation. This suggests that most psoriatic keratinocytes analyzed exhibited a cell intrinsic tendency toward Rac1 hyperactivation in response to various external stimuli, especially to those cytokines implicated in psoriasis pathogenesis.

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Overexpressing a Rac1V12 mutant under a keratin 14 promoter in a transgenic mouse model recapitulated many hallmarks of human psoriasis, including psoriasiform hyperplasia, a mixed inflammatory immune cell infiltrate, joint inflammation and a mutilating arthropathy. However, activated Rac1 in keratinocytes induced psoriasiform hyperplasia only in the presence of an intact immune system, as crossing Rac1V12 mice to T-cell deficient NOD/SCID mice, or treating Rac1V12 mice with immunosuppressive therapy (cyclosporine) rescued the psoriasiform phenotype. This indicated that activated Rac1 in the epidermis required immune derived stimulus, and that a skin specific defect can activate and differentiate the immune system resulting in systemic manifestations such as arthritis. Conversely, to assess whether immune derived stimulus required functional Rac1 in keratinocytes for psoriasis development, we isolated keratinocytes and fibroblasts from patient or control skin, and reconstructed organotypic 3D skin equivalents in vitro. This enabled selective modulation of Rac1 in keratinocytes before composition of skin grafts. These skin equivalents were then grafted to immunodeficient NOD/SCID mice, and after healing, grafts were intradermally injected with autologous PBMCs. Patient but not control xenografts developed profound psoriasiform hyperplasia, whereas selective inhibition of Rac1 in keratinocytes (by overexpressing a dominant negative mutant Rac1N17) rescued the phenotype. Thus, in human and murine skin, epidermal Rac1 activation is necessary to drive psoriasiform hyperplasia of the epidermis, but relies on immune derived factors. Intriguingly with increased Rac1 activity and mRNA expression in Rac1V12 skin, we found reduced levels of RhoA, and to a lesser extent CDC42. This indicates a coordinated regulation of RhoGTPases in the setting of activated Rac1 (and possibly other activated GTPases) in keratinocytes. In silico transcriptome analysis indicated that this could at least in part be due to enrichment of RhoGD1 signaling, which would be in agreement with previous studies on activated Rho mutants. This is especially interesting as RhoA has been shown to regulate epidermal differentiation, and we cannot exclude that the concomitant repression of RhoA could contribute to our findings as well as to the pathogenesis of psoriasis.

A hallmark of psoriatic lesional skin is perturbed skin barrier function, and it has been established that psoriatic skin exhibit increased trans-epidermal water loss, accompanied with reduced levels of proteins important for forming the cornified envelope of the epidermis. That genome wide association studies have implicated late cornified envelope proteins with psoriasis susceptibility indicates that perturbation of skin barrier integrity predispose to psoriasis. This is highlighted by the fact that minor skin trauma (a phenomenon called koeberization) is well known to induce psoriasis in those susceptible. A central transcription factor for regulating formation of the cornified cell envelope and keratinocyte differentiation is ZNF750. Our results indicate that activation of Rac1 reduces ZNF750 mRNA and protein expression, which would in turn lead to altered differentiation. Therefore, aberrant Rac1 activation in keratinocytes may have inhibitory effects on skin barrier integrity, through interfering with a key transcription factor underlying the terminal differentiation program in keratinocytes. Environmental stimulus such as wounding lead to Rac1 activation, and production of pro-proliferatory and pro-inflammatory cytokines such as interferons, TGFβ, IL36, CCL20, TNFα and others, which in turn may lead to a perturbed epidermal-immune signaling loop. This could further accentuate Rac1-stimulating factors through immune cell recruitment.

Rac1 cycles between a GDP-bound, inactivated, and GTP-bound, activated state, regulated by a complex network of Rac1-activating factors (GEFs), Rac1 inhibiting factors (GAPs) and RhoGD1 inhibitors (RhoGDIs). Several genetic association studies of psoriasis have implicated genes described previously to interact with Rac1, such as ZNF750, STAT3, IL36, CCL20, TNFα and others, as interferons, TGFβ, IL36, CCL20, TNFα and others, which in turn may lead to a perturbed epidermal-immune signaling loop. This could further accentuate Rac1-stimulating factors through immune cell recruitment.

Innate immune responses have been genetically linked with psoriasis, and encompass keratinocytes as well as immune cells. These signaling pathways could be important as to how genetic susceptibility of psoriasis could be associated with a predisposition to Rac1 hyperactivation in keratinocytes (and potentially other cell types). Interactions between the epidermis and the immune system are central to maintaining skin barrier homeostasis and is an essential aspect of the skin’s innate immunity. Responses involve sampling and sensing of antigens as well as production of antimicrobial peptides. It is tempting to speculate that Rac1 could play a role in early antigen sensing, thereby promoting immune cell recruitment. Perturbed antigen presentation has been implicated in psoriasis pathogenesis, and Rac1 has been demonstrated to play a role in antigen-presentation, at least in part through increased endocytosis. This is also an enriched pathway in the Rac1V12 mouse model of psoriasis. Other antigen sensing pathways in keratinocytes include pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRR); and keratinocytes may produce antimicrobial peptides.
such as β-defensins and cathelicidin LL-37 in response to stimuli. These interactions are an important step in innate immunity and the immune response. β-defensins are antimicrobial peptides readily produced by keratinocytes and have been found to increase Rac1 activation in skin, and another host defense peptide associated with psoriasis pathogenesis, cathelicidin LL-37, has also been shown to induce Rac1 activation. The specific mechanism whereby these peptides would activate Rac1 in keratinocytes remains unclear, but could encompass nonselective membrane receptors for cationic peptides such as the β defensins.

ELMO1 is a member of the engulfment and cell motility protein family, and is involved in innate immunity through TLR7- and TLR9-mediated IFN-α induction by plasmacytoid dendritic cells, ELMO1 is part of a Rac1-activating complex together with DOCK, and upon stimulation the ELMO-interacting region and the DOCK-homology region 2 guanine nucleotide exchange factor domain of DOCK2 interactions weakens their auto inhibition and induce Rac1 activation. This mechanism has been demonstrated in lymphocytes, although DOCK2 mediated signaling activating Rac1 has also been described in dendritic cells. As DOCK2 is mainly expressed in hematopoetic cells, the expression levels of this complex and relevance for keratinocyte mediated Rac1 activation remains to be elucidated. For instance, the ELMO-1 paralog ELMO-2 has been shown to involve epidermal growth factor mediated Rac1 activation in keratinocytes, and there DOCK proteins expressed in keratinocytes linked to Rac1 activation, such as DOCK1, indicating that this signaling axis could be relevant in increased Rac1 activation in psoriatic keratinocytes.

Besides aberrant activity Rac1 mRNA expression Rac1 is increased in psoriatic skin. Transcriptional regulation of Rac1 expression may therefore be involved in the deregulated Rac1-activation seen in psoriatic keratinocytes. It has been demonstrated in the nucleus accumbens the brain in a mouse model of stress and humans with depression that there is a sustained inhibition of Rac1 expression and a repressive chromatin state surrounding the proximal promoter of Rac1. Further, class 1 histone deacetylases (HDAC) rescued this repression as well as the phenotype in the mouse model. If the opposite mechanism with a activated chromatin state surrounding the promoter of Rac1 in susceptible cells is a feature of psoriatic patient cells, these cells could already be in an activated state when isolated from the skin. We cultured analyzed cells for several passages, and analyzed non-lesional skin keratinocytes to minimize confounding of previous activation states, but we do not have results definitely excluding that such a mechanism could persist. Thus, abnormal Rac1 activation could either occur through an intracellular signaling cascade, or indirectly through a feedback mechanism, possibly involving immune cell chemotaxis and cytokine production, promoting increase in Rac1-activating factors by both immune cells and keratinocytes.

It is also feasible that environmental stimulus or genetic susceptibility leads to an increased production of Rac1-activating factors by immune cells that once recruited into the skin potentiate and maintain a high level of Rac1 activation in the epidermis. Further, genetic factors promoting high Rac1 activity in keratinocytes could also predispose to activated Rac1 in other cell types. For instance, Rac1 has recently been demonstrated to drive interleukin 17 (IL17)
production in T-cells by forming a complex with RORγt and activate the IL17 promoter. IL17 is a key cytokine in psoriasis, pathogenesis, and stimulating keratinocytes with IL17 can lead to Rac1 activation in keratinocytes. This would manifest as high Rac1 activity in both keratinocytes and immune cells, and needs to be explored further. The fact the Rho-GTPase activating protein TAGAP involved in T-cell activation is genetically associated with psoriasis further implicates such a mechanism could be relevant. Altogether, understanding how increased Rac1 activation is induced and maintained in psoriatic cells will be central to understanding the mechanism of underlying disease-initiating factors (Fig. 1).

Several mechanisms are conceivable for the induced Rac1 activation in psoriatic lesional skin; including both increased Rac1-activating stimulus and intrinsic predisposition for Rac1 activation. This may include increased Rac1 GEF activity, diminished Rac1 GAP activity, altered binding to RhoGDI, or a spatially differential localization of Rac1 preventing its degradation or facilitating its persistent activation through differential GEF/GAP interactions. Understanding this may lead to development of targeted Rac1-inhibitory therapeutics in psoriasis.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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