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An "Ice-Cold" TR(i)P to Skin Biology: The Role of TRPA1 in Human Epidermal Keratinocytes

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Recent studies have suggested the expression of numerous heat-sensitive transient receptor potential (TRP) ion channels in non-neuronal cell populations of the skin. In this issue, Atoyan *et al.* provide evidence that the noxious cold-activated TRPA1 is widely expressed in various human cutaneous cells and that it may be directly involved in the regulation of keratinocyte proliferation and differentiation and in cutaneous inflammatory responses.

Journal of Investigative Dermatology (2009) **129,** 2096–2099. doi:10.1038/jid.2009.179

Thermosensitive transient receptor potential ion channels

Alteration in external temperature is a common challenge to homoeothermic organisms, initiating a coordinated response to maintain constant core temperature. Importantly, the sensory afferent as well as the majority of executive efferent mechanisms of the thermoregulatory response take place chiefly in the skin.

The key target molecules of temperature challenge are members of the large transient receptor potential (TRP)

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ion channel family (Clapham, 2003, Nilius *et al.*, 2007). Each of these distinct "cellular temperature sensor" molecules, originally identified in specific subsets of sensory afferent cells, is specialized to detect a well-defined temperature spectrum (Dhaka *et al.*, 2006). Indeed, certain nonselective cation channels that belong to the vanilloid (TRPV) subgroup are activated by warmth and heat, with the following activation ranges: ≥43 °C for TRPV1, ≥52 °C for TRPV2, ≥33 °C for TRPV3, and 27–42 °C for TRPV4 (Vriens *et al.*, 2009). Moreover, TRPM8 of the melastatin subfamily is activated by innocuous (i.e., not painful) coolness (≤28 °C), whereas TRPA1, a distant member of the TRP channel superfamily, is stimulated by noxious cold temperatures (≤17 °C) (McKemy *et al.*, 2002; Karashima *et al.*, 2009). Therefore, proper functioning of these nonselective cation channels enables the body to obtain temperature "readings" over almost the entire range faced by mammals.

The identification of plant-derived thermo-TRP-acting exogenous agents (e.g., capsaicin, camphor, menthol), as well as of numerous endogenous factors (e.g., inflammatory mediators, cytokines, protons, osmotic challenges, prostaglandins, leukotrienes, certain lipids, endocannabinoids) that activate and/or sensitize given TRP channels, uncovered the functional role of the thermo-TRPs in sensory mechanisms of the skin (Nilius *et al.*, 2007, Vriens *et al.*, 2009). Thermo-TRP channels were also implicated in cutaneous pain sensation, the development of pruritogenic itch, sensory neuron-derived neurogenic inflammation, and inflammatory thermal hyperalgesia (Caterina and Julius, 2001; Bíró *et al.*, 2007; Nilius *et al.*, 2007). Thus, thermo-TRPs are "multimodal" integrators in cutaneous sensory physiology.

TRPVs in non-neuronal skin cells: roles beyond the sensory

Recent data have suggested that the role of certain thermo-TRPs in the skin (and in other organs) is not restricted to sensory processes. Specifically, it has become clear that certain vanilloid thermo-TRPs are functionally expressed in numerous non-neuronal cell types of the skin. For example, the best-known member of the TRPV family, TRPV1 (the "capsaicin receptor"), was identified in epidermal and hair follicle keratinocytes, dermal mast cells, sebaceous gland–derived sebocytes, and dendritic cells (Inoue *et al.*, 2002; Bodó *et al.*, 2004; Ständer *et al.*, 2004). The activation of TRPV1, by altering gene expression profiles in the cells, suppressed *in vitro* human hair growth, inhibited proliferation of cultured human epidermal keratinocytes and sebocytes, induced apoptosis in a large variety of skin cells, and markedly affected such differentiation processes as lipid synthesis of sebaceous gland cells (Bodó *et al.*, 2005; Tóth *et al.*, 2009). In addition, a significant difference was observed in the *in vivo* hair follicle cycling of TRPV1 knockout mice when compared with their wild-type littermates (Bíró *et al.*, 2006).

Similarly, TRPV3 is highly and functionally expressed in epidermal and follicular keratinocytes (Peier *et al.*, 2002). Moreover, a constitutively active, "gain-of-function" mutation of the *trpv3* gene (*TRPV3*^{Gly573Ser}) in keratinocytes has been reported to be responsible for the development of a spontaneous hairless phenotype in DS-*Nh* mice (Asakawa *et al.*, 2006).

It has become evident that TRPV1 and TRPV3 are involved in the regulation of non-neurogenic inflammation. Specifically, activation of TRPV1 in human epidermal and hair follicle keratinocytes and in sebocytes markedly modulated the synthesis and release of pro- and anti-inflammatory cytokines, prostaglandins, and various growth factors (Southall *et al.*, 2003; Bodó *et al.*, 2005; Tóth *et al.*, 2009). Likewise, activation of TRPV3 in keratinocytes induced the release of proinflammatory interleukins and prostaglandins (Xu *et al.*, 2006; Huang *et al.*, 2008). Furthermore, DS-*Nh* mice with mutant TRPV3 and transgenic mice overexpressing the constitutively active mutant *trpv3* gene developed pruritic, atopic dermatitis-like skin alterations (Asakawa *et al.*, 2006; Yoshioka *et al.*, 2009).

Collectively, these findings suggest that certain thermo-TRPVs may be involved in nonsensory functions of the (human) skin, such as cutaneous growth control and immune/inflammatory processes. Furthermore, exogenous and endogenous (thermal, chemical) stimuli may similarly activate TRPVs expressed both in cutaneous sensory afferents and in non-neuronal skin cells. It should be noted, however, that most of the agents released from skin cells upon the activation of non-neuronal TRPVs (e.g., cytokines, prostaglandins) may in turn activate/sensitize TRPVs expressed in cutaneous sensory afferents. Therefore, it can also be postulated that non-neuronal skin cells, via the activation of their thermo-TRPVs and the release of "intercellular" messengers, may (at least indirectly) participate in cutaneous sensory processes such as thermosensation, nociception, and pruriception.

> Temperature-sensitive ion channels are expressed on non-neuronal cells in skin

TRPA1 is expressed in human non-neuronal skin cells and is implicated in a variety of cutaneous functions

TRPA1, a distant member of the TRP channel superfamily, was initially identified in polymodal sensory afferents (importantly, in colocalization with TRPV1) and was shown to be stimulated in the noxious cold temperature range (≤17 °C) (Clapham, 2003; Dhaka *et al.*, 2006). However, like thermo-TRPVs, TRPA1 can be activated by numerous natural compounds, including (i) pungent human skin irritants such as allyl isothyocyanate (alkaloids contained, e.g., in mustard oil, wasabi, and horseradish, that induce skin pain and inflammation similar to the TRPV1-activating capsaicin), (ii) cinnamaldehyde (a substance isolated from cinnamon oil) and allicin and diallyl disulfide (found in garlic), (iii) environmental irritants (e.g., acrolein), (iv) formalin, and (v) endogenous proalgesic inflammatory mediators such as bradykinin, arachidonic acid,

and prostaglandins (Dhaka *et al.*, 2006; Nilius *et al.*, 2007). Like TRPVs, TRPA1 was implicated not only in thermosensation but also in other cutaneous sensory neuron–coupled mechanisms such as pain sensation, hyperalgesia, and neurogenic inflammation (Dhaka *et al.*, 2006; Nilius *et al.*, 2007).

Intriguingly, an elegant study by Atoyan *et al*. (2009, this issue) introduces TRPA1 as an additional thermo-TRP "player" with roles beyond its sensory functions. Using immunofluorescence, Atoyan *et al*. report that TRPA1 is localized in various non-neuronal cell types in human skin *in situ*. Indeed, TRPA1 immunoreactivity was detected in the basal layer of the epidermis, the dermis, the epithelium of hair follicles, and pMel-17-positive epidermal melanocytes. Furthermore, the expression of TRPA1 (at both the protein and gene levels) was identified in primary cultures of human epidermal keratinocytes, melanocytes, and dermal fibroblasts, using western blotting and real-time PCR.

> Temperature-sensitive ion channels affect many functions of skin

To assess other functional roles for TRPA1, primary cultures of human epidermal keratinocytes were treated with the TRPA1 agonist icilin, and a comparative analysis of global gene expression profiles in keratinocytes (treated with icilin and vehicle control) was performed using a microarray platform and real-time PCR verification. The expression of 241 genes was regulated significantly by icilin treatment of keratinocytes. Intriguingly, the most prominent differences were observed in the expression of genes involved in the control of keratinocyte proliferation and differentiation and cell cycle regulation, including select members of the transforming growth factor-β superfamily (bone morphogenetic protein 7, growth differentiation factor 15), certain heat-shock proteins (HSP27, HSP90), regulatory factors stimulating keratinocyte differentiation, cyclins, and cyclin-dependent kinases and their inhibitors (cdc2, p21).

Significantly, gene expression analyses also revealed that activation of TRPA1 by icilin may not only modulate the delicate proliferation/differentiation program of keratinocytes but also affect keratinocyte-specific inflammatory responses. Treatment with icilin resulted in an increase in the expression of proinflammatory interleukins (IL-1α and IL-1β), suggesting that TRPA1 expressed in non-neuronal skin cells might be directly involved in the promotion of cutaneous inflammation.

Concluding remarks and perspectives

In summary, Atoyan *et al*. (2009) provide evidence that "ice-cold" TRPA1, like its "warm" and "hot" counterparts TRPV1 and TRPV3, is broadly expressed in non-neuronal cells of human skin and may be directly involved in the regulation of keratinocyte proliferation and differentiation, as well as in inflammatory responses. In addition, these data support the concept that epidermal keratinocytes (which express a variety of thermo-TRP channels) act in concert with sensory neurons (which express similar thermo-TRPs) to perceive the environment and to initiate protective cutaneous responses to environmental (thermal, chemical) stressors.

Targeted manipulation of certain thermo-TRP channels might be beneficial in addressing a multitude of human skin conditions, including cutaneous inflammation, hyperproliferative skin disease, and hair growth disorders. However, to predict the therapeutic potential of these exciting preclinical observations, numerous questions should be addressed with care:

- Does TRPA1 function as a nonselective cation channel on non-neuronal skin cells?
- Are TRPA1 (and TRPV) channels expressed in non-neuronal cutaneous cells in a fashion that is different from or similar to the TRP channels expressed in sensory neurons?
- Can cutaneous TRPA1 channels be activated by thermal and chemical stimuli?
- How do TRP-acting external stimuli affect skin cell proliferation, differentiation, immune competence/tolerance, and other processes?
- Is there any cross-talk between the coexpressed thermo-TRP channels and the related intracellular signaling pathways in cutaneous cells?
- Are there any alterations in the expression levels/patterns and functions of thermo-TRP channels in human dermatoses?

Targeting the cutaneous TRPs for therapeutic gain remains an intriguing and provocative possibility that warrants future study.

CONFLICT OF INTEREST
The authors state no conflict of interest.

ACKNOWLEDGMENT
This work was supported by the Hungarian Research Fund (OTKA K63153 and NK78398).

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