Gr-1+ cells from tumor-free mice, factors from melanoma and/or the melanoma microenvironment could be responsible for the selective expression of DC-HIL in melanoma and in MDSCs. The authors determined that IL-1β and IFN-γ, which were elevated in the B16-bearing mouse sera, could trigger DC-HIL expression synergistically. However, DC-HIL knockout mice showed similar kinetics of tumor growth compared with wild-type mice in EL-4 lymphoma and LL-2 lung carcinoma. Furthermore, MDSCs from these tumors did not show significant upregulation of DC-HIL and indeed demonstrated less suppressive activities. These observations, although implicating melanoma-specific mechanisms to promote DC-HIL expression and to foster MDSC functions, does not necessarily rule out a broader mechanism of action in other types of cancer.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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See related article on pg 2823

Newly Discovered Olfactory Receptors in Epidermal Keratinocytes Are Associated with Proliferation, Migration, and Re-Epithelialization of Keratinocytes

Mitsuhiro Denda[1,2]

Skin contains receptors for various environmental factors. In this issue of the Journal, Busse *et al.* cloned a new olfactory receptor, OR2AT4, in keratinocytes. They show that the activation of OR2AT4 induces phosphorylation of extracellular signal–regulated kinases and p38 mitogen–activated protein kinases, and that it accelerates wound healing. OR2AT4 may be a promising candidate as a target in clinical drug development.

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It is conceivable that chemoreceptor systems may have been present on the surface of the body early in the evolution of multicellular animals, because present-day invertebrates such as coelenterates, which originated during the pre-Cambrian period, have sensory and neural systems distributed throughout their bodies. Indeed, some vertebrates retain chemical receptors on their skin until they enter terrestrial life. For example, the odorant receptor family gene *OR2η4* is not expressed in adult frog skin, but it is expressed in larval skin (Amano and Gascuel, 2012). Thus, odorant receptors may have been expressed extensively on the skin surfaces of aquatic animals, but subsequently they may have been concentrated in olfactory cavities, after the animals had adapted to terrestrial life. In this scenario, it would not be surprising to find that some odorant and chemical receptors are still expressed in the skin of mammals. If so, would they retain functional roles?

We demonstrated previously that multiple transient receptor potential (TRP) channels are expressed in epidermal keratinocytes of mice and humans (Denda and Tsutsumi, 2011). We proposed that they formed part of a sensory system for physical and chemical factors

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Clinical Implications

- Drugs targeting OR2AT4 may promote wound healing.
- OR2AT4 and/or other sandalwood odorant receptors may be effective in cancer chemoprevention.
- Thus, functional olfactory receptors in epidermal keratinocytes may be significant in clinical dermatology, as well as in participating in whole-body responses to the environment.

in the environment. Among them, TRPV3 is activated and sensitized by plant odorants such as carvacrol, thymol, and eugenol (Xu *et al.*, 2006), which influence hair morphology and epidermal barrier homeostasis (Cheng *et al.*, 2010). Potentially, other chemical factors and receptors might be associated with epidermal homeostasis.

In this issue of the Journal, Busse *et al.* (2014) have shown that five olfactory receptors are expressed at the mRNA level in human keratinocytes. The investigators went on to clone and confirm functional expression of cutaneous olfactory receptor OR2AT4, and they then identified Sandalore, a synthetic sandalwood odorant, as an agonist of this orphan receptor (Busse *et al.*, 2014). Subsequently, they demonstrated that application of Sandalore induced the elevation of intracellular calcium and phosphorylation of extracellular signal–regulated kinases (ERKs) and p38 mitogen–activated protein kinases (MAPKs). Sandalore-induced signaling promoted proliferation and migration of keratinocytes. Immunohistochemical staining of human skin sections with an antibody against OR2AT4 (their Figure 2c) demonstrated the expression of OR2AT4 in keratinocytes at the basal layer, which is consistent with the association of OR2AT4 with keratinocyte proliferation. Indeed, they present convincing evidence that application of Sandalore accelerates the regeneration of cultured keratinocytes and accelerates wound healing of human skin *ex vivo* (their Figure 5e and f).

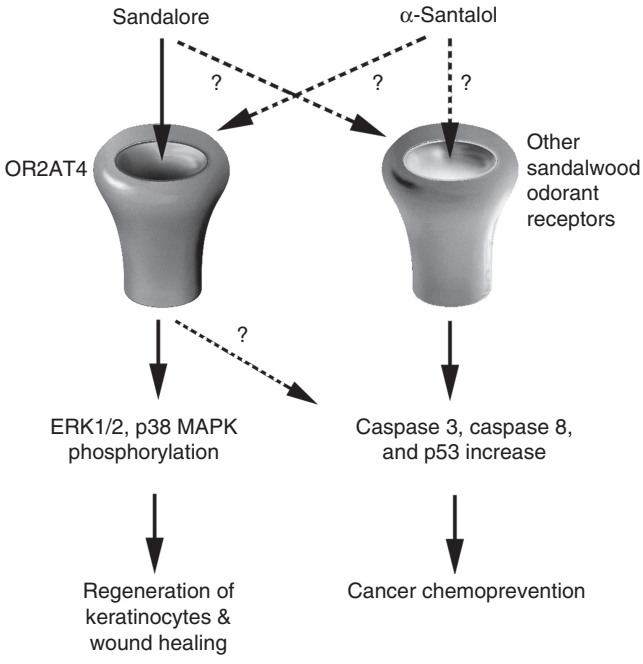

Figure 1. Schematic illustration showing signaling pathways that are potentially activated by sandalwood oil components and their putative effects. ERK, extracellular signal–regulated kinase; MAPK, mitogen-activated protein kinase.

These results suggest a new strategy for wound healing through targeting of OR2AT4.

An earlier review has concluded that sandalwood oil and its major component α-santalol have a cancer-chemopreventive effect (Zhang and Dwivedi, 2011). Topical application of sandalwood oil decreased the frequency of 7,12-dimethylbenz(a)anthracene (DMBA)–initiated and 12-O-tetradecanoylphorbol-13 acetate (TPA)–promoted skin tumors in CD-1 mice. Application of α-santalol also inhibited skin papilloma development induced by DMBA or TPA, and it reduced the multiplicity of tumors induced by UVB radiation. The mechanisms of these effects have been studied. For example, α-santalol caused cell cycle arrest at the G2/M phase, leading to the inhibition of cell proliferation, whereas proapoptotic proteins caspase-3 and caspase-8 and tumor-suppressor protein p53 were increased. These results suggested that activation of sandalwood-related receptors might be a good clinical strategy in treating skin cancer. Thus, identification of sandalwood receptor OR2AT4 is likely to be important not only for wound healing but also for the design of anticancer drugs. The findings described in this issue by Busse *et al.* (2014) are likely to stimulate additional studies of the role of OR2AT4 in cell signaling in the skin, as well as the search for other α-santalol-activated receptor(s) that might be associated with apoptosis-related proteins and p53.

It has been reported that olfactory simulation influences sleep time induced by pentobarbital administration in mice, and some odorants prolong sleep time, such as sedative drugs. These sleep-prolonging odorants may work by mitigating psychological stress. We have shown that psychological stress delays epidermal permeability barrier recovery after disruption (Denda *et al.*, 2000). Thus, we hypothesized that inhalation of such odorants might reduce the deleterious effect of psychological stress on barrier homeostasis. To test this hypothesis, we evaluated the barrier recovery rate after barrier disruption in both mice and humans under psychological stress (an unusual environment), in the presence and

absence of odorants that prolong sleep time. Indeed, we found that inhalation of these odorants restored the barrier recovery rate to the original, no-stress level (Denda *et al.*, 2000). In the human study, we placed a small cotton ball soaked with 50 μl of odorant-containing solution just beneath the nostrils and evaluated barrier recovery on the forearm. As this experiment was carried out in a large ($10 \times 8 \times 3$ m) room, it seems likely that most of the odorant would have been directly inhaled into the nostrils and very little would have diffused through the air and been directly absorbed at the forearm skin. On the other hand, in the mouse experiment, the odorant-containing cotton ball was simply placed in the small cage ($22.5 \times 33.8 \times 14.0$ cm). Thus, in the case of the mice, it is plausible that a significant proportion of the odorant molecules would have directly reached the skin surface, and it is conceivable that the odorant acted at least in part through unknown olfactory receptors expressed in mouse epidermal keratinocytes. This intriguing possibility seems worthy of further study. Comprehensive identification of olfactory receptors expressed in epidermal keratinocytes might be important clinically in relation to various skin diseases.

Not only odorants but also a variety of environmental factors influence epidermal homeostasis (Denda, 2012). For example, exposure to red light (wavelength: 550–670 nm) accelerated epidermal permeability barrier recovery after disruption, whereas exposure to blue light (430–510 nm) delayed it. We have also reported the expression of rhodopsin and opsins in human epidermis. In the retina, these photosensitive proteins have crucial roles in sensing brightness, darkness, and colors. On the other hand, sound at frequencies of 10, 20, and 30 kHz accelerates barrier recovery. Temperature and external electric potential also influence barrier homeostasis. Odorants influence barrier homeostasis and proliferation, migration, and regeneration of keratinocytes as well. Thus, sensory systems for many physical factors, such as visual, acoustic, and thermal factors, may have played important roles at the surface of the body early in evolution, as has been mentioned previously. In subsequent evolution in terrestrial environments, vertebrate skin became covered with squamous epithelium (reptiles), feathers (aves), or hair (mammals). Nevertheless, the work of Busse *et al.* (2014), Denda (2012), and others clearly indicates that at least some of the receptors are expressed and functional in the epidermis of mammals. Further, as humans (''naked apes'') have little hair, receptors in human skin might be activated by environmental factors much more readily than would be the case for other terrestrial animals. For example, Oohashi *et al.* (2006) have demonstrated that inaudible sound (at a frequency of more than 20 kHz) influences human brain-waves and plasma hormone levels. They have suggested that inaudible sound may be sensed at the surface of the body. In support of this concept, we have demonstrated that sound at 10–30 kHz accelerates barrier recovery and that it also accelerates the secretion of lamellar bodies in the uppermost layers of the epidermis (Denda, 2012). This gives rise to the idea that there might be an acoustic sensory system in epidermal keratinocytes.

In conclusion, the report by Busse *et al.* (2014) in this issue is of interest from the viewpoint of clinical dermatology, and OR2AT4 is a promising candidate as a target for clinical drug development. However, there may be more general implications, because environmental factors sensed at the surface of the skin are thought to influence not only skin physiology and pathology but also whole-body physiology and even emotion (Oohashi *et al.*, 2006). It has already been proposed that the epidermis represents the forefront of the body's systems for sensing the environment (Denda *et al.*, 2007). A comprehensive understanding of sensory receptors in epidermal keratinocytes could lead to a new paradigm of human healthcare in a changing environment (Figure 1).

CONFLICT OF INTEREST

The author states no conflict of interest.

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