COMMENTARY
include the majority of skin cancers (Nguyen and Atwood, 2018).

CONFLICT OF INTEREST
The authors declare no conflicts of interest.

REFERENCES

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Langerhans Cells Spy on Keratinocytes
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Tunneling nanotubes (TNTs) have been described as a novel mechanism for intercellular communication. However, the ability of epidermal cells to utilize TNTs remains a mystery. In this issue, Su and Igyártó (2019) showed that Langerhans cells (LCs) obtain mRNA from keratinocytes (KC) in vivo presumably via TNTs. The demonstration of exchange of genetic material from KC to LC in vivo is an unexpected method of antigen acquisition by LC and also an important consideration when interpreting transcriptomic data.


Cell-to-cell communication is fundamental for the maintenance of tissue homeostasis and the execution of efficient physiological responses to an array of stimuli. Hemopoietic and non-hemopoietic cells accomplish this task by secreting and responding to soluble ligands (e.g., cytokines and hormones), through gap junctions, extracellular vesicles (exosomes and microvesicles), and tunneling nanotubes (TNTs) (Mittal et al., 2019; van Niel et al., 2018). The latter two mechanisms transfer a large diversity of molecular cargo (e.g., DNA, protein, lipids, and mRNA) that is not secreted normally. Since their recent discovery, TNTs have been reported to be utilized by epithelial cells and myeloid cells (e.g., monocytes, macrophages, and dendritic cells) (Dupont et al., 2018; Rustom et al., 2004). However, the ability of epidermal Langerhans cells (LCs) to use TNTs to communicate with keratinocytes (KCs) or dendritic epidermal T cells (DETCs) had yet to be explored. In a report in the Journal of Investigative Dermatology, Su et al. provide evidence that LCs have the ability to exchange mRNA to other epidermal cells, presumably through TNTs (Su and Igyarto, 2019).

Su and colleagues began by carefully analyzing transcriptomic datasets provided by the IMMGEN Genome project, a scientific collaboration that collects and curates transcriptomic data from flow cytometry–purified mouse immune cells. They noted the presence of several KC-specific transcripts such as keratins (e.g., K14, K10, and K5) in epidermal LCs. This phenomenon had been noted in unpublished work by several groups, but it was attributed to potential cross-contamination of KCs with LCs during sorting experiments. Su and colleagues (2019) dug deeper and confirmed that epidermal LCs contained KC-specific mRNA transcripts. LCs contained easily detectable levels of keratin mRNA, but chromatin analysis of LCs clearly indicated that K14 and other keratin gene loci were unavailable, indicating that LCs were not actively transcribing K14. Despite not actively transcribing keratins, keratin protein was evident in LCs. These data suggest that LCs can acquire mRNA from KCs, but a more formal demonstration required a clever but complex experimental approach. To accomplish this, the authors created K14-YFP mice in which the K14 promoter drives the expression of Cre recombinase in a ROSA26.YFP fate reporter mouse, resulting in YFP expression in KCs. To ensure that Cre and YFP expression are absolutely excluded from LCs, K14-YFP mice were bred to huLangerin-DETA mice that lack LCs. These mice then were transplanted with bone marrow from wild-type (WT) mice to replenish the mice with WT LCs. This resulted in mice where Cre and YFP are robustly expressed by KCs, and there is no possibility that they are expressed by LCs. Despite the absence of YFP

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transcription in LCs, YFP mRNA and protein were detected in LCs. Moreover, LCs were able to acquire Cre itself from KCs, resulting in genetic recombination in LCs. Together, these data nicely demonstrate that LCs have the capacity to acquire mRNA from KCs in functionally relevant quantities. In addition, YFP expressed only in LCs could be observed in DETCs and KCs, suggesting bidirectional transfer of cargo (Figure 1).

To address the question of how LCs acquire cargo from KCs, Su and colleagues next examined in vitro transwell experiments. Sorted LCs and KCs were unable to transfer cargo when physically separated, suggesting that soluble structures such as exosomes are not sufficient. They also show that YFP acquired from KCs in vivo is localized throughout the cytoplasm in LCs and not in vesicles. This argues against phagocytosis as a potential mechanism.

TNFAIP2 (also known as B94 or M-sec) is a cytosol protein that has been showed to participate in TNT formation by inducing F-actin membrane protrusions (Hase et al., 2009). LCs are the only cell type in the epidermis that expresses TNFAIP2. This is consistent with previous studies that showed that TNFAIP2 is predominantly expressed in myeloid cells and not by epithelial cells (Hase et al., 2009). Based on this, the authors suggest that LCs may acquire cargo from KCs via TNTs. Confirmation of this will require future experiments with mice defective in TNT formation.

The possibility of TNT formation in the epidermis is appealing because TNTs have been described as contributing to disease pathogenesis by allowing the transfer of viruses, misfolded proteins (prions), and cancer-promoting cellular material (Mittal et al., 2019). If transferred via TNTs, pathogenic material avoids exposure to the extracellular space and thus is not accessible to antigen-presenting cells. These observations raise questions about the role of epidermal TNTs in cutaneous diseases and whether TNTs can be exploited as therapeutic targets to treat cutaneous diseases.

A number of interesting possibilities arise from the discovery that LCs acquire antigens from KCs. There are strong data indicating that LCs present endogenous antigens on major histocompatibility complex I (MHC-I) (Kaplan, 2017). The question of whether or not LCs have the capacity to cross-present acquired antigens on MHC-I has been the source of a long-simmering debate with conflicting data. The observation that LCs can acquire a cytosolic source of antigen from KCs that presumably would be presented on MHC-I may provide some clarity to this debate. Presentation of self-antigens by DCs during steady-state conditions provides maintenance of central tolerance (Steinman and Nussenzweig, 2002). Acquisition of KC mRNA by LCs via TNTs (or other transfer mechanisms) would provide LCs with a broader repertoire of KC antigens than could be obtained by traditional methods of antigen uptake. This may increase the efficiency of peripheral tolerance. Though LCs may assist in this process, it is likely to be redundant, as LC-deficient mice do not develop autoimmunity and skin migratory DCs as a group are sufficient to promote self-tolerance (Nirschl et al., 2017). Transfer of KC mRNA to LCs may also play an important role in immunosurveillance against keratinocyte neoplasia.

Perhaps the greatest immediate impact from this work is the lesson that genes identified by transcriptomic analysis may not actually be transcribed by the cells under study. Unexpected genes associated with unrelated cell types are routinely found in RNA sequencing studies. In many circumstances, these observations are thought to reveal novel, unexpected biology. This study cautions that in some circumstances, the presence of genes associated with an unrelated cell type may in fact may have been acquired from adjacent cells, and this possibility should not be overlooked.

Clinical Implications

Langerhans cells can obtain keratinocyte mRNA that may promote self-tolerance and tumor immunosurveillance.

Figure 1. Transfer of functional mRNA between epidermal cells. Model scheme showing that Langerhans cells exchange mRNA transcripts and proteins with keratinocytes and DETCs, presumably via tunneling nanotubes. DETC, dendritic epidermal T cell.
CONFLICT OF INTEREST
The authors state no conflict of interest.

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