Title
Tight junction properties change during epidermis development.

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regulation of catagen formation in the hair follicle (16). IL-6, which shows upregulation in balding DPCs, compared with non-balding DPCs, may be a key factor in inhibition of hair growth. Anagen-to-catagen transition, characterized by apoptotic cell death in epithelial cells in the hair bulb and outer root sheath, is driven by factors such as TGF-β1 and TGF-β2 (17–20). Recombinant human DKK-1 has been reported to inhibit growth of ORSCs and trigger apoptotic cell death (21). IL-4, IL-6, TGF-β1, TGF-β2 and DKK-1 can be expressed in cultured sebocytes.

Involvement of several members of the FGF family in regulation of the hair growth cycle has also been reported (10,22). Both FGF-1 and FGF-2 stimulated DPCs to release factors that induce DNA synthesis in ORSCs. FGF-5 has been reported to inhibit hair growth and induce catagen in hair follicles of mice and to attenuate DPCs-mediated ORSCs proliferation through blockade of the effect of FGF-1 (23). FGF-5 is known to be associated with reduction in hair density.

In this study, treatment with NSS-conditioned medium resulted in a decrease in the survival rate of ORSCs and DPCs and inhibition of hair growth. Gene expression of Wnt10b, Lef1, TGF-ß1 and TGF-ß2 showed a decrease in cultured NSS. In addition, increased gene expression of FGF-5, which was also revealed by microarray, as well as IL-4, IL-6 and DKK-1, was observed in cultured NSS.

The authors believe that differences in expression of bioactive markers of NSS from NS may be a causative factor in inhibition of terminal hair growth. In addition, cellular activities and expression of bioactive markers of conventional two-dimensional cell culture differ from those of three-dimensional culture; therefore, further studies using three-dimensional NSS culture are needed to achieve better understanding of hair growth inhibition in nevus sebaceus (24).

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Author contributions

Weon Ju Lee and Hyun Jung Lim conducted the research; Weon Ju Lee, Seok-Jong Lee and Do Won Kim designed the research study; Weon Ju Lee and Hyun Wuk Cha performed data analysis; and Weon Ju Lee and Hyun Wuk Cha prepared the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Primers of the biomarkers.

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Letter to the Editor

Tight junction properties change during epidermis development

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Experimental Dermatology

Abstract: In terrestrial animals, the epidermal barrier transitions from covering an organism suspended in a liquid environment *in utero*, to protecting a terrestrial animal postnatally from air and environmental exposure. Tight junctions (TJ) are essential for establishing the epidermal permeability barrier during embryonic development and modulate normal epidermal development and barrier functions postnatally. We now report that TJ function, as well as claudin-1 and occludin expression, change in parallel during late epidermal development. Specifically, TJ block the paracellular movement of Lanthanum (La^{3+}) early in rat *in vivo* prenatal epidermal development, at gestational days 18–19, with concurrent upregulation of claudin-1 and occludin. TJ then become more permeable to ions and water as the fetus approaches parturition, concomitant with development of the lipid epidermal permeability barrier, at days 20–21. This sequence is recapitulated in cultured human epidermal equivalents (HEE), as assessed both by ultrastructural studies comparing permeation of large and small molecules and by the standard electrophysiologic parameter of resistance (R), suggesting further that this pattern of development is intrinsic to mammalian epidermal development. These findings demonstrate that the role of TJ changes during epidermal development, and further suggest that the TJ-based and lipid-based epidermal permeability barriers are interdependent.

Abbreviations: TJ, tight junctions; TER, transepithelial resistance; ENaC, epithelial sodium channel; SC, stratum corneum; SG, stratum granulosum; HEE, human epidermal equivalents; TEWL, transepidermal water loss.

Key words: epidermal development – epidermal permeability barrier – tight junction – transepithelial resistance

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Background

Epidermis must transition from a prenatal epithelium, in which regulated water and ion flux may be beneficial, to a postnatal epidermis that must provide an essentially impermeable barrier to water, ions and toxins or bacteria. Defective epidermal permeability function is devastating, especially for premature infants (<33 weeks gestation), whose skin cannot yet protect against water, calorie and electrolyte loss (15,17) or sepsis owing to microbial invasion (22).

The relative roles of tight junctions (TJ) and the lipid-based barrier in maintaining the epidermal permeability barrier have been the subject of recent intense interest (24), with some studies supporting a primary role for the lipid-based barrier in postnatal epidermis (2,3,7–13,18,19,25,27), while others show that TJ are essential for perinatal survival and normal epidermal function (4,14,21,23,26,28–31).

Questions addressed

We hypothesized that TJ form the major water and ion barrier early in development and that this function changes when the lipid barrier is established. Further, we hypothesized that the barrier function of TJ would change during development, blocking water and ions early, but only larger molecules once the lipid barrier was in place.

Experimental design

Rat fetuses were harvested from day 17 to day 22 of gestation. Cell culture, immunoblotting, electron microscopy, light and confocal microscopy were performed using standard methods (see Supporting Information).

Results

TJ Expression and function change during rat embryonic development

Mirroring mice and humans (5), the rat epidermal lipid-based barrier consistently develops late in rat gestation, around gestational day 20–21 (rats are born gestational day 22) (1,16). Relative claudin-1 and occludin protein expression levels peaked at day 18/19, then decreased at days 20–21 (Fig. 1a), the period during which the lipid barrier is established (16). La^{3+}, an electron dense element with a hydrated radius (0.4 nm) similar to that of Na^{+} (0.3 nm), was blocked at sites of TJ in the SG at day 18 (Fig. 1b and Figures S1a,b) but permeated through TJ sites in the SG and was blocked instead at the location of the epidermal lipid barrier, at the base of the SC, after the lipid-based permeability barrier was formed postnatally (Fig 1c and Figure S1d). Secreted lipid processed into bilayers was noted in postnatal epidermis (16), denoting a functional lipid barrier in this epidermis. These experiments demonstrate that TJ were able to block ion and water flux through the epidermis transiently *in utero*, but lost this ability late in gestation. Conversely, a lipid-based barrier was not formed early in gestation, but developed late in gestation and was able to block ion and water flux postnatally.

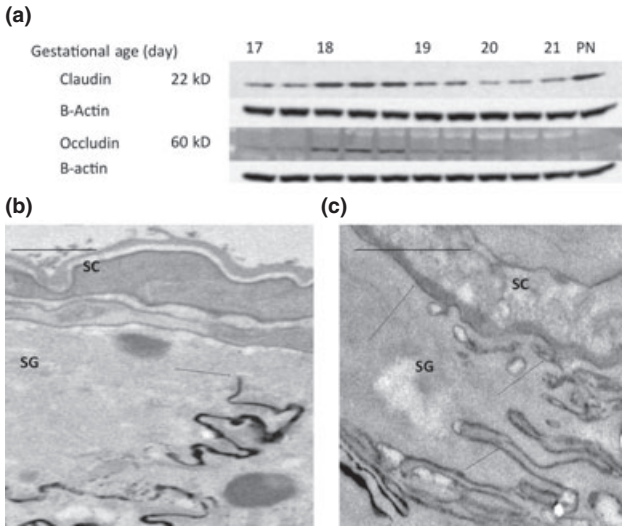

Figure 1. Tight Junction Formation in *in vivo* Rat Fetal Development. (a) Tight Junction protein expression in epidermis during the perinatal period. Western blots demonstrate that both claudin-1 and occludin expression peak at gestational day 18/19 and diminish as the fetal rat barrier is formed (day 20) and approaches parturition (day 22). Claudin-1 then peaks postnatally (PN), while occludin remains low. (b) La^{3+} permeation at fetal day 18. La^{3+} permeates the viable epidermis until its diffusion is blocked by TJ between the lateral borders of the SG cells (arrow). (c) La^{3+} permeation at postnatal day 3. In contrast to panel B, La^{3+} permeates through the lateral borders of the SG (arrows) and is blocked not at the SG, but instead is blocked at the SG/SC interface. SC = Stratum Corneum. SG = Stratum Granulosum. N = 2–3 pups. Scale bar = 1 μm.

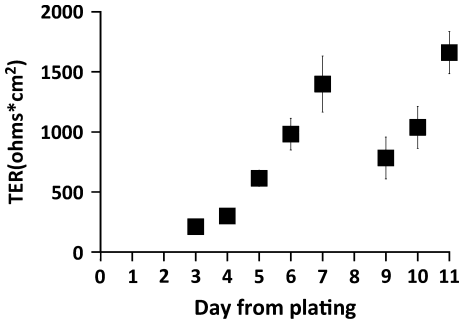

Figure 2. Transepithelial Resistance measurements in Developing human epidermal equivalents (HEE). Transepithelial resistance (TER) was measured in HEE cultured with those analysed in Figure S1. An initial maximum is recorded at day 7. TER then drops until day 9 and increases again until day 11. n = 9–36 for each time point. Data are presented as the mean ± SD.

TJ Changes are recapitulated in a human epidermal equivalent model

Human epidermal equivalents (HEE) are useful models of epidermal differentiation, as they reproduce both the epidermal differentiation and the lipid barrier seen in skin and can be used for electrical measurements, because they do not contain hair follicles, eccrine glands or dermis.

Morphology and TJ protein expression was similar in HEE and rat fetal epidermis, with development of a functional lipid-based barrier by 11–12 days (Figure S2). EM micrographs revealed structures typical of tight junctional complexes in cultures at days 5–6 (Figure S3). La^{3+} perfusion was blocked at TJ sites in the SG at days 5–6 (Figure S2c and S3), when relative claudin-1 and occludin expression was high (Figure S2b), corresponding with days 18–19 in rat skin. Likewise, La^{3+} permeated through these sites and was instead blocked at the SG/SC interface by the lipid barrier at day 11 (Figure S2f and S4), as seen in postnatal rat skin (compare to Fig. 1c). Because La^{3+} permeation cannot measure the global permeability barrier function of the epidermis, we additionally measured electrical parameters (6). Transepithelial resistance (TER) peaked at day 7 (Fig. 2), when La^{3+} permeation was blocked at TJ sites (Figure S2c). TER dropped precipitously until day 9, corresponding to decreases in occludin expression. However, TER then peaked again at day 10–11 (Fig. 2), correlating with the development of a SC, secreted and processed lipid, and a competent lipid-based barrier that blocked La^{3+} permeation at the SC/SG interface (Figure S2g).

TJ Block paracellular movement of macromolecules later in development

TJ have been noted to block larger molecules, such as biotin, in postnatal epidermis (20). HEE impeded passage of biotin at TJ sites, even as they no longer blocked La^{3+} flux (Figure S5). These experiments suggest that TJ function changes as the epidermis matures. The evolution of TJ permeability likely corresponds to different physiologic requirements for TJ's at various stages of epidermal development.

Conclusions

TJ are essential for establishing the epidermal permeability barrier during embryonic development and modulate normal epidermal development and barrier functions postnatally. TJ block the paracellular movement of Lanthanum (La^{3+}) early in rat *in vivo* prenatal epidermal development and early in HEE differentiation, concurrent with upregulation of claudin and occludin. TJ then become more permeable to ions and water as the lipid epidermal permeability barrier develops. However, TJ continue to block paracellular access by large molecules, even though they become permeable to ions, suggesting an important role for these structures in postnatal epidermis. These findings demonstrate that the role of TJ changes during epidermal development and further suggest that the TJ-based and lipid-based epidermal permeability barriers are interdependent.

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Author contributions

Celli A: Performed research, designed experiments, analysed data, wrote manuscript. Zhai Y: Performed research, designed experiments, analysed data. Jiang YJ: Performed research. Crumrine D: Performed research, analysed data. Elias PM: designed research, analysed data, wrote manuscript. Feingold KR: designed experiments, provided reagents. Mauro TM: designed experiments, analysed data, wrote manuscript.

Conflict of interests

The authors have declared no conflicting interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Tight Junction Formation *in vivo* Rat Fetal Development.

Figure S2. Tight Junction Formation in Developing HEE.

Figure S3. Lanthanum perfusion in developing HHE.

Figure S4. Lanthanum perfusion in mature HHE.

Figure S5. TJ impede biotin permeation in fully developed HEE.

Figure S6. Kissing points and TJ structures are present in Day 6 Lifted Cultures.

Figure S7. Kissing points and TJ structures are present in Day 6 Lifted Cultures.

Data S1. Materials and Methods.

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