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Eccrine sweat gland development and sweat secretion

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Abstract

Eccrine sweat glands help to maintain homeostasis, primarily by stabilizing body temperature. Derived from embryonic ectoderm, millions of eccrine glands are distributed across human skin and secrete litres of sweat per day. Their easy accessibility has facilitated the start of analyses of their development and function. Mouse genetic models find sweat gland development regulated sequentially by Wnt, Eda and Shh pathways, although precise subpathways and additional regulators require further elucidation. Mature glands have two secretory cell types, clear and dark cells, whose comparative development and functional interactions remain largely unknown. Clear cells have long been known as the major secretory cells, but recent studies suggest that dark cells are also indispensable for sweat secretion. Dark cell-specific Foxa1 expression was shown to regulate a Ca^{2+} -dependent Best2 anion channel that is the candidate driver for the required ion currents. Overall, it was shown that cholinergic impulses trigger sweat secretion in mature glands through second messengers – for example InsP3 and Ca^{2+} – and downstream ion channels/transporters in the framework of a $\text{Na}^+\text{-K}^+\text{-Cl}^-$ cotransporter model. Notably, the microenvironment surrounding secretory cells, including acid–base balance, was implicated to be important for proper sweat secretion, which requires further clarification. Furthermore, multiple ion channels have been shown to be expressed in clear and dark cells, but the degree to which various ion channels function redundantly or indispensably also remains to be determined.

Keywords

Best2; Ca^{2+} ; clear cell; dark cell; ectodysplasin; FoxA1; InsP3R2; Itpk; Nkcc1; Shh; Wnt

Introduction

Two major types of sweat glands, apocrine and eccrine, are known in humans. Apocrine glands are a phylogenetic remnant of mammalian scent glands. They are larger but are non-thermoregulatory restricted into a few areas, notably the axillae and external genitalia (1). They secrete turbid fluid containing water, proteins, lipids and odour precursors with a DNA variant in the *ABCC11* required for odour formation (2). By contrast, eccrine glands are tiny but very numerous (3). Millions are distributed across the human skin and directly open to

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Conflict of interests

The authors have declared no conflicting interests.

the skin surface. Eccrine glands form a thermoregulatory organ and secrete primarily water that contains electrolytes. We focus on the eccrine glands in this review.

An individual can secrete up to 4 l of eccrine sweat in an hour (3), cooling down body temperature as necessary. Evaporation of sweat from the skin surface effectively dissipates heat generated by physical activity, fever or hot environments. Dysfunction of sweat glands, seen characteristically in the elderly, is linked to the heat stroke that kills about 1000 people each year in the United States (www.cdc.gov/mmwr). By contrast, overactive sweat glands can cause local hyperhidrosis, a chronic condition affecting the quality of life of about 2.8% of the US population (4).

Eccrine glands also have additional roles in humans. They secrete moisturizing factors such as lactate, urea, sodium and potassium to maintain skin hydration (5). Further, secreted sweat mixed with sebum on the skin surface forms a moisturizing lipid layer (6). Recent studies have further demonstrated that sweat glands secrete several antimicrobial peptides, including dermcidin, cathelicidin and lactoferrin (7–10), which help to control skin flora and fight skin infections. Furthermore, sweat also contains secreted IgA and cytokines, including IL-1 and IL-31, which likely contribute to immune defense and the extent of inflammatory reactions (11–14).

Thus, increasing evidence suggests critical involvement of eccrine glands in skin homeostasis as well as thermoregulation. Here, we review recent findings on the mechanism of eccrine gland development and secretion. Other aspects, including sweat gland-related diseases and therapeutic approaches, are covered in extensive reviews (1,3,15–21).

Morphological features of eccrine sweat glands

Each eccrine gland is a single tubular structure, about 3–5 mm in length in humans with a secretory coil below a relatively straight sweat duct (Fig. 1) (17). The secretory coil contains three types of cells – dark, clear and myoepithelial – identifiable by conventional microscopy in humans. Dark and clear cells are secretory cells, whereas myoepithelial cells support the glands mechanically and create a major niche for gland stem cells (3,18,22). Dark cells contain cytoplasmic electron-dense granules and secrete periodic acid–Schiff (PAS)-positive diastase-resistant glycoproteins as well as the novel antimicrobial dermcidin (7,23). Notably, dark cells have relatively few mitochondria and membrane villi and were long thought to play a minor role in sweat secretion (3) (but see below). By contrast, clear cells have many mitochondria and membrane villi and have major secretory function, extending to excessive sweat production in patients with local hyperhidrosis (24).

Among other markers, S100 and CA II are expressed only in clear cells, CGRP mainly in dark cells, Krt5/Krt14 in myoepithelial cells and Krt8/Krt18 in both dark and clear cells (25–28). These proteins are thus useful for immunostaining to distinguish the different cell types (Table 1). In addition, several ion channels, pumps and cotransporters were identified in human dark and/or clear cells. The NKCC1, Na-K-ATPase, NHE1, CFTR and AQP5 are all expressed in the secretory coil (29–36) (Table 1).

The sweat duct connects the secretory coil to the skin surface. The duct itself consists of two layers of cuboidal cells, suprabasal (luminal) and basal (Fig. 1). The basal cells strongly express several ion channels and transporters including CFTR, ENaC, Na-K-ATPase and more weakly NKCC1 and NHE1 (3,29–31,37), suggesting they are involved in the partial reabsorption of ions during the excretion process (Table 1). Lesions in the chloride channel CFTR cause cystic fibrosis, an autosomal recessive life-threatening disease affecting lungs, pancreases and sweat glands (38).

Regulation of eccrine sweat gland development

Origin and timing

Like all skin appendages, eccrine glands are derived from embryonic ectoderm. The eccrine glands form everywhere in human skin apart from a few areas such as lips and external auditory canals. In most other mammals, eccrine glands are located only in ventral paw skin, typically clustered in contact areas such as dome-shaped footpads. Accordingly, humans cool down body temperature by sweating, whereas other mammals generally conserve heat by hair coat and dissipate heat in alternative less efficient ways such as panting, increased saliva secretion and elevated blood flow in less hairy skin. In this sense, expanded skin coverage by sweat glands (eccrine) is an evolutionary marker, with the sole known exception of ungulates, in which apocrine rather than eccrine glands increase to numbers comparable to our species. In humans, eccrine glands begin to form around the 4th gestational month in palmoplantar skin and at the 5th month across the rest of the body, maturing morphologically before term. In mice, eccrine gland formation in ventral paw skin begins around E16.5–E17.5 and is completed around postnatal day 21 (39,40).

Dermal signalling centre

Skin appendage formation is characteristically regulated by reciprocal signalling interactions between ectoderm and mesenchyme in general, and hair follicles, teeth, mammary glands and salivary glands all have clearly visible dermal papillae or dermal condensations below epidermal structures (41,42). A long-standing puzzle in morphological features of developing eccrine glands was the lack of a corresponding histologically discernible dermal condensation or any dermal signalling centre (43). However, we have found that mouse eccrine gland germs are nevertheless surrounded by clustered mesenchymal cells (dermal sheath) during development, although the sheath was small (40). The small size likely accounts for the failure of conventional detection, but the notion is thus supported that eccrine gland formation requires an interaction of ectoderm with mesenchyme. The identity and function of eccrine gland mesenchymal cells remain to be characterized.

Involvement of Wnt–Eda–Shh cascade and Bmp

Signalling pathways are correlated with morphological stages in sweat gland formation. A Wnt/ β -catenin–Eda/NF- κ B–Shh cascade functions in a genetic relay for eccrine gland development. The process starts with K14-positive progenitor cells in epidermis, as seen in mouse models (22). Morphogenetic pathways similar to those seen for other skin appendages are involved in the regulation of early-stage eccrine gland development (40). Wnt signalling is required for eccrine gland induction. As in hair follicles, uniform Wnt

activity can be detected in dermis before any morphological indication of eccrine gland induction, but declines quickly as the gland germs start to form. Instead, at this stage, strong Wnt activity appears in the basal layer of epidermis, gradually concentrating in the gland germs. Wnt is then continuously active at the tip of growing ducts until it disappears as sweat ducts start to coil. Consistent with its requirement for induction, inactivation of Wnt signalling by β -catenin knockout in mouse skin epidermis results in total absence of eccrine gland induction, and overexpression of Dkk4, a Wnt antagonist, significantly blocks eccrine gland induction (40).

Directly activated by Wnt, the Eda pathway regulates the next stage of development. When the cytokine Eda or its receptor Edar or adaptor Edaradd is inactive, Lef1-positive pregerms still form but never develop to full germs (40,44). Furthermore, sweat ducts fail to form if *Eda* is selectively inactivated after the germ formation stage (45). By contrast, supplementation of the Eda-A1 isoform rescues eccrine gland formation in *Eda*-null Tabby mice (46).

The next stage of secretory portion formation requires Shh signalling. Unlike its early involvement in hair follicle development (47,48), Shh inactivation does not affect eccrine gland germ or subsequent duct formation, but the secretory coil formation remains blocked at a rudimentary stage (40,49).

This signalling cascade, however, has additional actors. One additional critical morphogen is Bmp: overexpression of the Bmp antagonist noggin in skin epidermis led to hair follicle formation in mouse footpads, which are otherwise the exclusive site for eccrine sweat glands (50). In a recent study, eccrine glands were shown to fail to form in *Bmpr1a* knockout mice (51) although detailed analysis was not included. Suppression of the Bmp pathway was reported to promote hair follicle formation (52). However, eccrine gland formation may still require active Bmp signalling, a possibility that requires further experimental testing.

Innervation

Innervation, an obvious indispensable requirement for regulation of sweat secretion, develops along with the sweat glands. In normal mouse development, sympathetic innervation starts in developing eccrine glands at postnatal day 1 (P1) – the early secretory coil formation stage – and transition to cholinergic innervation can be detected at P6, when secretory coil formation is far along (53). Sympathetic nerve fibres were found distributed in the ‘correct’ location even when eccrine glands were missing, although they disappeared after a short period of about 2 weeks (54). The mature glands are then surrounded by postganglionic sympathetic nerve endings, although the principal final neurotransmitter is acetylcholine (3). Notably, nascent fibres failed to acquire cholinergic status in the absence of association with developing eccrine glands (54). Thus, the final transition to cholinergic response likely requires humoral factors released by developing eccrine glands (55–57). Notably, NGF transgenic mice showed a lack of eccrine gland innervation, although sympathetic axons reached the footpads, implicating NGF as a participant in innervation (58). A further indication of its importance in eccrine gland innervation comes from findings that mutations in NGF receptor TrkA led to apoptosis of primary afferent and postganglionic

sympathetic nerves during development, resulting in congenital insensitivity to pain with anhidrosis (59).

Eccrine sweat gland progenitor/stem cells

Sweat gland progenitor/stem cells were recently identified from developing and mature mouse eccrine glands by lineage tracing (22). Eccrine gland formation starts with K14+ multipotent bud progenitors in the basal layer of embryonic ectoderm. The K14+ bud progenitors then transition to transient multipotent K14+ basal progenitors and K18+/lowK14 suprabasal progenitors as development proceeds. Eventually, they form four unipotent adult progenitor/stem cell populations in mature glands: basal duct, suprabasal duct, myoepithelial, and glandular luminal progenitors (22). Consistent with their regenerative potential, the basal and suprabasal duct progenitors were shown to contribute to wound healing of epidermal sweat duct and skin epidermis. Further, myoepithelial and glandular luminal progenitors replenished their own descendants only when localized injury was made, suggesting their unipotent characteristics. However, some unipotent progenitors likely regain multipotent status when localized in foreign environments. The myoepithelial and basal duct progenitors, but not luminal or suprabasal duct progenitors, were shown to regenerate de novo sweat glands in mammary and shoulder fat pads and stratified epidermis in back skin (22). Among the four adult progenitor populations, myoepithelial and basal duct progenitors, but not luminal and suprabasal duct progenitors, form large colonies and can be cultured long term (22). Notably, the myoepithelial progenitors were shown to be the major 'label-retaining cells' (22,51) and can regenerate sweat glands and hair follicles in foreign environments (51). Therefore, myoepithelial progenitors may possess greater 'stemness' and greater potential to regenerate sweat glands or skin epidermis.

Human eccrine sweat gland epithelial cells have also been shown to regenerate epidermis during skin wound healing (60,61). Human eccrine gland stem cells have not yet been characterized in detail, but myoepithelial cells were suggested to be the progenitor cells (62).

Mechanism of sweat secretion

Neuronal control

Sweat secretion in mature eccrine glands is controlled by the central nerve system. Several types of sweating are regulated at different levels of neuronal centres (16). Cortical sweating responds to emotions and is seen on palms and soles. Medullary sweating responds to spicy food and is found on the face. But hypothalamic sweating, the thermoregulatory mediator, responds to elevated body temperature. It is systemic and predominant. The thermosensitive neurons (warm-sensitive and cold-sensitive) are located in the preoptic area of the hypothalamus, and local heating of that area induces sweating (63). Eccrine sweat glands respond to both core (internal) and peripheral (skin) temperatures. The internal temperature is much more efficient than skin temperature for both sweat induction and sweating rate (64,65); this may reflect the steadier internal signal compared to external cold or hot objects or conditions.

Innervation by cholinergic sympathetic nerves is consistent with reports that denervation, like postganglionic sympathectomy, abruptly abrogates sweating and is widely used clinically to treat local hyperhidrosis (66). Sweating can occur when periglandular cholinergic, α -adrenergic or β -adrenergic nerves are activated, but cholinergic sweat secretion is the major route and also accounts for more than 70% of sweating capacity in isolated human eccrine sweat glands analysed in vitro with pharmacological agonists (Table 2) (3). In fact, inhibition of beta-adrenergic nerve activity had no detectable effect on sweat secretion in people during heat acclimation (67). Furthermore, eccrine glands express adrenergic $\alpha 1$ and $\beta 2$ receptors particularly during the transition of noradrenergic to cholinergic innervation at early developmental stages, but they decline sharply at adult stages, consistent with the ineffective sweating induced by adrenergic agonists (68,69). Therefore, adrenergic sweating in physiological conditions is questionable and of unknown significance.

Released acetylcholine (ACh) functions in eccrine glands through its receptor Chrm3, and not via M1 or M2 types (70,71). Chrm3 is expressed in the secretory cells as well as in myoepithelial cells, and blocking Chrm3 with specific antagonists inhibited sweat secretion (72,73). Some *Chrm3* mutations cause the prune belly-like syndrome in human (OMIM#100100) (74), and *Chrm3* mutant mice showed similar phenotypes, including decreased salivary secretion (75). The secretory function of eccrine glands in *Chrm3* mutant patients and mice has not been documented in detail, but they are predicted to be hypohidrotic (75) (Table 3).

Two neuropeptides have been suggested as further regulators of sweat secretion. Galanin increased the chloride current in NCL-SG3 sweat gland cells, and a GalR3 antagonist blocked the current (76); and both galanin and its receptors GalR2 and GalR3 are expressed in human eccrine glands. Galanin knockout sweat glands, however, showed normal innervation and normal response to sudorific agents, and thermal stimulation rather increased the number of active eccrine glands compared to wild-type mice (72). Furthermore, galanin transgenic mice showed normal sweating (77), and therefore, any substantive galanin function in sweat secretion remains uncertain. A second neuropeptide, CGRP, is also itself expressed in dark cells, and enhanced acetylcholine-induced sweating in humans when applied dermally (78), but no further studies of its mechanism or relative importance have been reported.

Ca²⁺ requirement

Ca²⁺ is unequivocally required for sweat secretion (79). The immediate response to cholinergic input on eccrine glands is a sharp increase of cytosolic Ca²⁺. This is accomplished from two sources: influx from extracellular interstitial fluid and release of intracellular Ca²⁺ stores. Intracellular Ca²⁺ release is mediated at least in part by InsP3. When acetylcholine activates the Chrm3 receptor, InsP3 is produced from PIP2 in the secretory cells, most likely through phospholipase C (PLC) action, which then promotes intracellular Ca²⁺ release from the endoplasmic reticulum (3) [similar to observations in kidney epithelial cells (80)]. A recent genetic study showed that mutations in InsP3R2 blocked Ca²⁺ release in the secretory cells and resulted in severe hypohidrosis both in

patients and in a knockout mouse model (81). Thus, an Ach-Chrm3-PLC-InsP3-InsP3R2 pathway is instrumental for intracellular Ca^{2+} mobilization. It remains to be seen whether elaborated InsP3 and increased Ca^{2+} in activated secretory cells can spread to neighbouring secretory cells, as they do in airway epithelial cells (82), to trigger sweat secretion.

The other source of Ca^{2+} , influx from extracellular fluid, is also prerequisite for sweat secretion. Muscarinic sweating was completely blocked when Ca^{2+} was removed from the culture medium of isolated monkey eccrine glands (79), and local chelation of Ca^{2+} in humans resulted in a significant decrease in cholinergic sensitivity (83). The mechanism of Ca^{2+} influx into sweat secretory cells is poorly understood, but involvement of voltage-gated Ca^{2+} channels has been suggested (79,83). An intriguing candidate effector to be explored as a second messenger is InsP4, derived from InsP3 by Itpks. InsP4 (and not InsP3) was reported to induce Ca^{2+} influx into neurons, involving voltage-gated calcium channels (84–86), and we have found that Itpks are expressed in mouse eccrine glands (work in progress). Speculatively, a balance between InsP3 and InsP4 in secretory cells could thus vary the relative extent of intracellular Ca^{2+} release and extracellular Ca^{2+} influx to optimize sweat secretion (Fig. 2a).

InsP4 in conjunction with InsP3 was further shown to activate Ca^{2+} -dependent K^+ channels to induce an outward K^+ current in lacrimal acinar cells, and also enhanced Ca^{2+} -activated chloride current in pancreatic cells (87,88). Given that K^+ and Cl^- efflux is critical for sweat secretion as well, InsP4/InsP3 may function similarly on Ca^{2+} -activated K^+ and Cl^- channels in sweat glands. It may be relevant that elevation of Ca^{2+} in the sweat secretory cells, induced by a calcium ionophore, does not itself induce sweat secretion. Rather, sweating was induced when Ca^{2+} concentration was extremely high, but at even higher levels, above those that muscarinic agents can attain, sweating again failed (89). This would be consistent with a requirement for increased production of InsP3/InsP4 by muscarinic stimulation not only for elevation of Ca^{2+} concentration in secretory cells, but also for effective Ca^{2+} action on K^+ and Cl^- channels in sweat secretion.

Na^+ - K^+ - Cl^- cotransporter model

Cholinergic sweat secretion has been correlated with a Na^+ - K^+ - Cl^- cotransporter model, as has also been suggested for secretory processes in salivary glands and pancreas (3). In this model, acetylcholine release from periglandular sympathetic nerves activates K^+ and Cl^- channels of secretory cells, most likely via the action of second messengers (79,89), leading to efflux of K^+ ions into the interstitium and Cl^- ions into the secretory lumen. The chemical gradient formed by K^+ and Cl^- efflux would subsequently activate the Nkcc1 cotransporter, the major ionic force, resulting in influx of Na^+ , K^+ and Cl^- ions into secretory cells in an electrically neutral way. Indeed, when Nkcc1 was inhibited, sweat secretion ceased abruptly (90). Excessive Na^+ ions entering the secretory cells would then be successively pumped out to the interstitium, drawn into the secretory lumens by Cl^- ions – plausibly through tight junctions between secretory cells – and followed by water – perhaps through AQP5 water channel (32,33) – to form nearly isotonic primary sweat. Partial reabsorption of NaCl in the sweat duct at the subsequent excretion stage results in final hypotonic sweat.

The Na⁺-K⁺-Cl⁻ cotransporter model can explain most features of cholinergic sweat secretion. However, among many missing details, the identity of the ion channels involved and their relative roles remain conjectural. Several candidate ion channels have recently been identified in sweat glands. TMEM16A, a CaCC, and its splice isoform, TMEM16A(*ac e3*), are expressed in human sweat glands and contribute to Cl⁻ secretion in NCL-SG3 cells (91). The *ac e3* isoform was implicated in basal sweat secretion because it was active even in the absence of Ca²⁺ influx. We have recently identified a number of K⁺ and Cl⁻ channels from mouse sweat glands, and Ca²⁺-activated Kcnn4 and pH-sensitive Kcnk5 channels were suggested to be partially required for sweat secretion (Cui CY & Schlessinger D, unpublished data).

Involvement of dark cells and Best2 in sweat secretion

Unlike clear cells, involvement of dark cells in sweat secretion is not well characterized. We summarize the available information and outline some alternative possibilities as follows. It was suggested that dark, clear and myoepithelial cells, all derived from embryonic ectoderm, require one another to maintain their identity, as they lose their morphological characteristics quickly when cultured in vitro (3). Consistent with this phenomenon, the origin of NCL-SG3, the only cell line derived from human sweat glands, was unidentifiable and in fact was shown to respond to β -adrenergic rather than the expected physiological cholinergic stimulation (92). Notably, as in clear cells, the dark cells were also shown to change cellular ion concentrations drastically upon methacholine stimulation (93), and at least some dark cells were shown to have many mitochondria and membrane villi (3). Thus, in addition to secreting glycoproteins and antimicrobial dermcidin, dark cells may play an important role for sweat secretion, assisting clear cells. Recent findings of a Foxa1–Best2 cascade have more decisively suggested involvement of dark cells in sweat secretion (94). Both Foxa1 and Best2 were expressed exclusively in mouse dark cells, and *Foxa1* knockout mice showed anhidrosis, while *Best2* knockout mice showed severe hypohidrosis (Table 3). Best2 was shown to function in HEK-293 cells as a CaCC that is also permeable to other anions including HCO₃⁻ (95,96). However, it functions exclusively as a HCO₃⁻ channel *in vivo* in mucin-producing goblet colon cells (97). It is still to be determined whether Best2 is a HCO₃⁻ channel or a Cl⁻ channel, or transports both as in cell culture system in glycoprotein-producing dark cells (95,96). But if it is a HCO₃⁻ channel as in goblet cells, increased Ca²⁺ ions in dark cell cytosol, induced by cholinergic inputs, could activate Best2 to transport HCO₃⁻, which may contribute to formation of a proper extracellular or intracellular microenvironment to foster secretion.

Coincidentally, dark cells are known to secrete sialomucin, an acidic adhesive glycoprotein, which is confined to the intercellular canaliculi and luminal surface of secretory cells, the critical structures for sweat secretion (98). It is conceivable that the microenvironment, including acid–base balance, at ion channel/transporter sites, is important for sweat secretion. We suggest as a working model that HCO₃⁻ ions secreted by Best2 channels may be involved in generating a balanced extracellular environment by cooperating with acidic proteins such as sialomucin (Fig. 2b). Consistent with the notion of acid–base regulatory involvement, pH-sensitive alkaline phosphatases were detected in the intercellular canaliculi of sweat gland secretory cells (99), and inactivation of alkaline phosphatases resulted in Cl⁻

secretion through the CFTR chloride channels in human airway epithelial cells (100). More recently, a missense mutation in the *CA XII* in an autosomal recessively inherited family was shown to cause excessive Na^+ secretion in sweat (101). *CA XII* is a transmembrane protein with an extracellular active site that interconverts carbon dioxide and bicarbonate. *CA XII* was suggested to be broadly expressed in sweat glands (102) and could thus affect the extracellular microenvironment for sweat secretion and reabsorption. In addition, it was previously shown that inhibition of the $\text{HCO}_3^-/\text{Cl}^-$ (or Na^+/H^+) exchange alone, without blocking of Best2 channels or other transporters, partially reduced K^+ efflux and sweat secretion in isolated monkey sweat glands (3,90).

An alternative possible mode of action for Best2 is suggested by the demonstrated effects of cytosolic pH on ion channel activity. K^+ current was increased when intracellular pH was elevated in rabbit colon cells or rat pancreatic B cells (103,104). Similarly, basic cytosolic pH activated and acidic pH inhibited ENaC sodium channel in human sweat ducts (105). Therefore, secretion or influx of HCO_3^- by Best2 may affect intracellular acid–base balance and ion channels/transporters in the secretory coil. Intracellular or extracellular acid–base changes by Best2 may also affect pH-sensitive ion channels in sweat glands – for example *Kcnk5*, which is localized in dark cells in mice (Cui CY & Schlessinger D, unpublished data). The hypohidrosis seen in *Best2* knockout mice might then result from an inadequate extracellular or intracellular microenvironment, and the anhidrosis found in *Foxa1* knockout mice could be due to lack of Best2 as well as significant reduction of *Nkcc1* expression in sweat glands (94).

The routes to intracellular regulation of sweat secretion are of course not limited to direct effects on ion channels. For example, PAR-2 is also expressed in NCL-SG3 cells, and activation of PAR-2 by trypsin-like serine proteases increased Ca^{2+} concentrations in cytosol and enhanced chloride secretion (106). In addition, histamine was recently shown to inhibit sweat secretion by suppressing phosphorylation of *GSK3 β* , which is a target for phosphorylation by acetylcholine (107,108). This may hint at an uncharacterized mechanism involving glycogen levels in sweat secretion.

Major open questions

We have described first steps in the analysis of sweat gland development and function that raise many questions. We suggest four that are ripe for investigation, primarily in the mouse model system. First, what is the regulatory network regulating the secretory machinery? Genetic and epigenetic regulation of secretory function should be assessed (109). *Foxa1* is the first transcription factor identified in sweat glands, known to regulate Best2 anion channels, but many more must be characterized. Also missing from present formulations is the ‘fine-tuning’ of regulation of gene expression by a cohort of non-coding RNAs in developing and mature sweat glands (110,111). Large-scale genomic and proteomic profiling should increasingly provide a full spectrum of gene expression and its variation. Second, dark cells are implicated in sweat secretion, but what is the detailed process – including the origins, interdependency, and functional interactions of clear and dark cells? For example, as an alternative to be tested, chemical gradients formed by $\text{Cl}^-/\text{HCO}_3^-$ secretion in dark cells by Best2 could then spread to clear cells through gap junctions to

maximize sweat secretion (Fig. 2b). A technical challenge to the analyses is presented by the lack of known reliable cell membrane markers that could permit isolation of the cell types for direct analyses. The hurdle could be resolved with extended analyses of cell composition. Third, what second messengers mediate Ca^{2+} influx from extracellular fluid upon cholinergic impulses? Fourth, which of the many ion channels in eccrine sweat glands are indispensable, and how and in which cells? An adjunct to the identification of critical components for questions 3 and 4 could be targeted analyses of mouse genetic models and human diseases, which have largely ignored in the examination of sweat glands (Table 3).

Perspectives

As mentioned above, signalling pathways for development and ion channels/transporters for secretion are known to overlap for several exocrine glands. Although the specific fate decisions and secretions of each gland obviously require specific study, the structurally comparably simple eccrine sweat glands could provide a model for features of dynamics of other exocrine glands as well. Answers to the major open questions posed above can fill in many critical details for comparative analysis. Furthermore, they are the prelude to further analyses of the way in which eccrine gland dynamics – from the impulse provided by sympathetic nerves to the composition and amount of sweat produced – change with genetic variation and with age. Such analyses can provide a route to understand congenital eccrine gland agenesis, local hyperhidrosis, and ageing-related hypohidrosis.

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Abbreviations

AQP5	aquaporin-5
CaCC	Ca^{2+} -activated chloride channel
CA II	carbonic anhydrase 2
CA XII	carbonic anhydrase 12
CFTR	cystic fibrosis transmembrane conductance regulator
CGRP	calcitonin gene-related peptide
Chrm3	acetylcholine muscarinic receptor M3
ENaC	epithelial sodium channel
InsP3	inositol 1,4,5-trisphosphate
InsP3R2	InsP3 receptor type 2
InsP4	inositol 1,3,4,5-tetrakisphosphate

Itpks	InsP3 kinases
Na-K-ATPase	Na ⁺ -K ⁺ pump
NGF	nerve growth factor
NHE1	Na ⁺ /H ⁺ exchanger
NKCC1	Na ⁺ -K ⁺ -Cl ⁻ cotransporter 1
PAR-2	proteinase-activated receptor 2

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Mouse eccrine gland

Figure 1.

A mouse eccrine sweat gland and cellular constituents are depicted. As labelled, the eccrine gland comprises a secretory coil in the deep dermis and a relatively straight duct open to the skin surface (left panel). The secretory coil contains three types of cells, clear, dark and myoepithelial, all derived from embryonic ectoderm. Clear and dark cells are secretory cells, and myoepithelial cells form a major niche for sweat gland stem/progenitor cells (right lower panel). The sweat duct consists of suprabasal (luminal) and basal layers where ions partially reabsorbed (right upper panel).

Figure 2.

Working models for calcium action and dark cell function in sweat secretion. Panel a: A possible cascade of Ca²⁺ release and influx. Acetylcholine binds to Chrm3 receptor and activates phospholipase C (PLC) to generate InsP₃ from PIP₂. InsP₃ in turn activates InsP₃R2 in the endoplasmic reticulum to release Ca²⁺ from reserves (blue). At the same time, InsP₃ can be phosphorylated by Itpks to generate InsP₄, which may induce Ca²⁺ influx from extracellular interstitium (red). These events most likely occur in clear cells (81), but it remains to be seen whether InsP₃ and P₄ spread to dark cells. Panel b: Possible involvement of dark cells in sweat secretion. Dark cell-specific Best2 may be a HCO₃⁻ and Cl⁻ channel (see text). Elevated Ca²⁺ ions (red) activate Best2 (blue) and could secrete HCO₃⁻ and/or Cl⁻ into the extracellular region. HCO₃⁻ may then affect acid–base balance and the functions of channels and transporters in intercellular canaliculi and at the luminal surface, interacting with proteins such as acidic sialomucin. CA XII may also be involved in the formation of a pH gradient and thereby affect the function of alkaline phosphatases. In parallel, Cl⁻/HCO₃⁻ secretion may create a chemical gradient in dark cells that spreads to clear cells through gap junctions, and could thereby maximize sweat secretion in cooperation with clear cells.

Table 1

Cellular constituents and known key regulators of human eccrine sweat glands

Anatomical structures	Cellular constituents	Key proteins
Sweat duct	Basal cells	CFTR, ENaC, Na ⁺ -K ⁺ -ATPase, NKCC1 (weak), NHE1, K1, K10, K5, K14
	Suprabasal (luminal) cells	CFTR, NHE1, K1b, K10, K19, K77
Secretory portion	Dark cells	NKCC1, Na ⁺ -K ⁺ -ATPase, NHE1, CFTR, AQP5, CGRP, K8, K18
	Clear cells	NKCC1, Na ⁺ -K ⁺ -ATPase, NHE1, CFTR, AQP5, S100, CA II, K8, K18
	Myoepithelial cells	K5, K14

Table 2

Pharmacological reagents commonly used for studies of sweat secretion

Pharmacological reagents	Function
Methacholine	Muscarinic receptor agonist
Pilocarpine	Muscarinic receptor agonist
Carbachol	Muscarinic and nicotinic receptor agonist
Atropine	Muscarinic receptor antagonist
Phenylephrine	α -adrenergic receptor agonist
Isoproterenol	β -adrenergic receptor agonist
Forskolin	cAMP inducer
Bumetanide	NKCC1 inhibitor
Ouabain	Na^+/K^+ -ATPase (Na^+/K^+ pump) inhibitor
Amiloride	ENaC sodium channel inhibitor
Ba^{2+}	K^+ channel inhibitor
Niflumic acid	Ca^{2+} -activated Cl^- channel inhibitor
NPPB	Cl^- channel inhibitor
DIDS	$\text{Cl}^-/\text{HCO}_3^-$ exchanger inhibitor
A23187	Ca^{2+} carrier (ionophore)

Table 3

A list of genes and their animal models suggested in sweat secretion

Genes	Genotype	Gross phenotype	Sweat gland phenotype	References
<i>Foxa1</i>	cKO (skin)	None	Anhidrosis	(94)
<i>Best2</i>	KI	None	Hypohidrosis	(94,112)
<i>Itpr2</i> (InsP3R2)	KO	None	Hypohidrosis	(81)
<i>Aqp5</i>	KO	None	Hypohidrosis; Normal sweating	(32,33)
<i>Chrm3</i>	KO	Reduced body weight, dilated pupils	Not analysed	(75,113)
<i>Slc12a2</i> (Nkcc1)	KO	Deafness, head-shaking, growth retardation, circling	Not analysed	(114–116)
<i>Atp1a1</i> (Na ⁺ -K ⁺ pump)	KO	KO perinatal lethal. Heterozygotes viable	Not analysed	(117)
<i>Cftr</i>	KO	KO poor growth, decreased survival, intestinal obstruction	Not analysed	(118–122)
	cKO			
<i>Scnn1a</i> (ENaC)	KO	KO perinatal lethal	Not analysed	(123)
	cKO			
<i>Slc9a1</i> (Nhe1)	KO	2/3 of progeny die before weaning	Not analysed	(124)