Acta Histochemica 117 (2015) 275–278

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Combination of keratins and alpha-smooth muscle actin distinguishes secretory coils from ducts of eccrine sweat glands

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ARTICLE INFO

Article history:
Received 25 January 2015
Received in revised form 5 March 2015
Accepted 6 March 2015

Keywords:
Eccrine sweat glands
Secretory coil
Duct
Keratins
Alpha-SMA

ABSTRACT

Eccrine sweat glands are comprised of secretory coils and ducts, which are distinct in morphology and function. To better understand the roles of the two parts in development, homeostasis, wound repair and regeneration of eccrine sweat glands, we must distinguish between them. In this study, the localization of keratins and alpha-SMA in human eccrine sweat glands was examined by immunofluorescence staining. Based on the differential localization of keratins and alpha-SMA in different cell types, four pairs of antibodies (K5/K7, K5/alpha-SMA, K14/K7 and K14/alpha-SMA) were used to differentiate secretory coils from ducts by double-immunofluorescence staining. Immunofluorescence staining showed that myoepithelial cells of secretory coils expressed K5, K14 and alpha-SMA, whereas secretory cells of secretory coils expressed K7, K8, K15, K18 and K19. Ductal cells expressed K5, K8, K14 and K19. Double-staining showed that the secretory coils were $K5^+/K7^+$, $K5^+$/alpha-SMA^+, $K14^+/K7^+$ and $K14^+$/alpha-SMA^+, whereas ducts were $K5^+/K7^-$, $K5^+$/alpha-SMA^-, $K14^+/K7^-$ and $K14^+$/alpha-SMA^-. In conclusion, by combining use of keratins and alpha-SMA antibodies, secretory coils can be easily differentiated from ducts in morphology.

Introduction

Eccrine sweat glands are distributed in almost all human skin, where they play important roles in regulating human body temperature by the evaporation of sweat (Rittie et al., 2013; Saga, 2002). Eccrine sweat glands are simple tubular glands, composed of two distinct parts: secretory coils and ducts. The secretory coils are a pseudostratified epithelium, consisting of a discontinuous outer basal layer of myoepithelial cells and an inner suprabasal layer of secretory cells (Saga, 2002). Myoepithelial cells are located between the secretory cells and the basement membrane, and interdigitate with the secretory cells (Saga, 2002). The ducts are stratified epithelium, which are composed of two layers of cells: the peripheral ductal cells and the luminal ductal cells (Saga, 2002). The duct comprises a coiled duct, an intradermal straight duct, and an intraepidermal duct (Saga, 2002). Morphologically, it is often difficult to differentiate between secretory coils and ducts.

Secretory coils and ducts are distinct in function. The secretory coils secrete isotonic fluid into the lumen from which the ducts reabsorbed sodium, thereby modifying sweat from a basically isotonic solution to a hypotonic one (Saga, 2002). Ductal and secretory progenitors differ not only in normal homeostasis but also in their response to different types of tissue injuries (Lu et al., 2012). Myoepithelial progenitors of secretory coils only replace damaged myoepithelial cells, secretory progenitors of secretory coils only replace damaged secretory cells and ductal progenitors participate in epidermal wound repair, which have been demonstrated in the mouse paw (Lu et al., 2012). Both the secretory coils and ducts have the ability to form functional skin substitutes, but their potential in epidermal regeneration is different, with secretory coils being

Abbreviations: Alpha-SMA, alpha-smooth muscle actin; BSA, bovine serum albumin; DAPI, 4′,6-diamidino-2-phenylindole; MC, myoepithelial cells; PBS, phosphate buffer saline.

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http://dx.doi.org/10.1016/j.acthis.2015.03.004
0065-1281/

superior over ducts (Pontiggia et al., 2014). The secretory cells show a higher proliferative and colony-forming efficiency, more successful epidermis formation, and thicker newly formed epidermis than ductal cells (Pontiggia et al., 2014).

To better understand the roles of secretory coils and ducts in the development, homeostasis, wound repair and regeneration of eccrine sweat glands, secretory coils must be distinct from ducts. Noël et al. (2013) and Pontiggia et al. (2014) study the differential expression of single marker in absorptive and secretory cells to distinguish between the two portions. In this study, the localization of keratins and alpha-SMA in human eccrine sweat glands was examined by immunofluorescence staining. Based on the differential localization of keratins and alpha-SMA in different cell types, four pairs of antibodies (K5/K7, K5/alpha-SMA, K14/K7 and K14/alpha-SMA) were used to differentiate secretory coils from ducts by double-immunofluorescence staining. By combining use of keratins and alpha-SMA antibodies, secretory coils can be easily differentiated from ducts in morphology.

Materials and methods

Skin samples

Full-thickness skin specimens were obtained from individuals undergoing plastic surgery in the Burn and Plastic Surgery Department of the Second Affiliated Hospital of Shantou University Medical College. The average age was 35.2 ± 3.4 years and the regions from which the specimens were derived included palms, foot, arms and legs. Ethical permission was granted by the Ethics Committee of Shantou University Medical College (Shantou, China), and informed consent was obtained from patients or their guardians. Twelve skin samples were fixed in 4% paraformaldehyde, paraffin-embedded and cut into 5 μm-thick serial sections.

Immunofluorescence staining of the eccrine sweat glands

Immunofluorescence staining was performed as follows. First, sections were deparaffinized and rehydrated. Second, the sections were heated to 95 °C in 0.01 M citric acid buffer (pH 6.0) for 15 min, and then slowly cooled to room temperature for antigen retrieval. Third, the sections were incubated with 1% BSA in PBS for 30 min at 37 °C to block nonspecific sites. Forth, the sections were incubated respectively with the following primary antibodies at 1:100 dilution: rabbit anti-K5 (ZA0518, ZSGB-BIO, Beijing, China), mouse anti-K7 (ZM0071, ZSGB-BIO, Beijing, China), mouse anti-K8 (ZM0310, ZSGB-BIO, Beijing, China), rabbit anti-K14 (ZA0540, ZSGB-BIO, Beijing, China), rabbit anti-K15 (AB52816, Abcam, Cambridge, UK), mouse anti-K18 (ZM0310, ZSGB-BIO, Beijing, China), mouse anti-K19 (ZM0074, ZSGB-BIO, Beijing, China) and mouse anti-alpha-SMA (BM0002, Boster, Wuhan, China), at 4 °C in the dark overnight. For sections incubated with the mouse primary antibodies, ALex FLuor488-labeled secondary antibody (A0428, Beyotime, Jiangsu, China) was used, and Cy3-labeled (A0516, Beyotime, Jiangsu, China) or ALex FLuor488-labeled (A0423, Beyotime, Jiangsu, China) goat anti-rabbit secondary antibodies were used for sections incubated with rabbit primary antibodies. For all secondary antibodies incubations was for 1 h at room temperature in the dark at 1:500 dilution. Finally, sections were counterstained with 5 μg/ml DAPI (Beyotime, Jiangsu, China) for 10 min at room temperature in the dark and mounted with antifade mounting medium (Beyotime, Jiangsu, China). PBS was used for rinsing between steps. Sections omitting the primary antibodies were used as negative controls. The sections were viewed with a fluorescence microscope (Olympus BX51, Tokyo, Japan). Immunofluorescence staining was evaluated independently by two pathologists. All the used primary antibodies were company validated for fluorescence immunohistochemistry on formalin fixed tissues.

Table 1
Differential expression of keratins and alpha-SMA in secretory coils and duct cells of eccrine sweat glands.

Antibody	Secretory coils		Ducts	
	Secretory cells	MC	LC	PC
K5	–	+	+	+
K7	+	–	–	–
K8	+	–	+	–
K14	–	+	+	+
K15	+	–	–	–
K18	+	–	–	–
K19	+	–	+	+
Alpha-SMA	–	+	–	–

MC, myoepithelial cells; LC, luminal duct cells; PC, peripheral duct cells; +, expressed; –, not expressed.

Double-immunofluorescence staining of the eccrine sweat glands

The protocols for double-immunofluorescence staining were the same as for immunofluorescence staining except simultaneous incubation with both primary antibodies (mouse anti-K7/rabbit anti-K5, mouse anti-K7/rabbit anti-K14, mouse anti-alpha-SMA/rabbit anti-K5 and mouse anti-alpha-SMA/rabbit anti-K14) and simultaneous incubation with both secondary antibodies together (Alexa FLuor488-labeled goat anti-mouse IgG and Cy3-labeled goat anti-rabbit IgG) occurred.

Results

Differential expression of keratins and α-SMA in different eccrine sweat gland cell types

The expression patterns of keratins and alpha-SMA are consistent in all the skin samples evaluated. Keratins and alpha-SMA were expressed in the cell cytoplasm (Fig. 1). MC of secretory coils expressed K5 (Fig. 1A1–3), K14 (Fig. 1D1–3) and alpha-SMA (Fig. 1H1–3). Secretory cells of the secretory coils expressed K7 (Fig. 1B1–3), K8 (Fig. 1C1–3), K15 (Fig. 1E1–3), K18 (Fig. 1F1–3) and K19 (Fig. 1G1–3). Ductal cells expressed K5 (Fig. 1A1–3), K8 (Fig. 1C1–3), K14 (Fig. 1D1–3) and K19 (Fig. 1G1–3), with K8 only reacting with luminal cells (Fig. 1C1–3). The staining pattern of K5, K7, K8, K14 and K19 in the coiled dermal duct, straight dermal duct and intraepidermal duct were similar. The differential expression of keratins and alpha-SMA in secretory coils and duct cells of eccrine sweat glands were reported in Table 1. In the case of negative control, there was no positive staining.

Distinguishing between secretory coils and ducts

According to the specific expression patterns of keratins and α-SMA in different eccrine sweat gland cell types, we used the following four pairs of primary antibodies to distinguish coils from ducts: K7/K5, K7/K14, alpha-SMA/K5 and alpha-SMA/K14. Double-immunofluorescence staining showed that secretory coils were positive for K7/K5 (Fig. 2A1–5), K7/K14 (Fig. 2B1–4), alpha-SMA/K5 (Fig. 2C1–4) and alpha-SMA/K14 (Fig. 2D1–4); ducts were positive for K5 (Fig. 2A1, A4, A5, C1, C4, C5) and K14 (Fig. 2B1, B4, B5, D1, D4, D5), but were negative for K7 (Fig. 2A2, A4, A5, B2, B4, B5) and alpha-SMA (Fig. 2C2, C4, C5, D2, D4, D5).