Differential antigen expression between human apocrine sweat glands and eccrine sweat glands

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Research Article

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Abstract

Bromhidrosis has a great negative impact on personal occupation and social psychology. It is not yet clear whether bromhidrosis is caused by apocrine sweat glands or the co-action of apocrine sweat glands and eccrine sweat glands. To distinguish between apocrine sweat glands and eccrine sweat glands, specific antigen markers for apocrine sweat glands and eccrine sweat glands must be found first. In the study, we detected the expression of K7, K18, K19, Na⁺-K⁺-2Cl⁻ cotransporter 1 (NKCC1), carbonic anhydrase II (CAII), Forkhead transcription factor a1 (Foxa1), homeobox transcription factor engrailed homeobox1 (En1), gross cystic disease fluid protein-15 (GCDFP-15), mucin-1 (MUC-1), cluster of differentiation 15 (CD15) and apolipoprotein (APOD) in eccrine sweat glands and apocrine sweat glands by immunofluorescence staining. The results showed that K7, K18, K19, Foxa1, GCDFP-15 and MUC-1 were expressed in both apocrine and eccrine sweat glands, CD15 and APOD were only expressed in apocrine sweat glands, and CAII, NKCC1 and En1 were only expressed in eccrine sweat glands. We conclude that CD15 and APOD can serve as specific markers for apocrine sweat glands, while CAII, NKCC1 and En1 can serve as specific markers for eccrine sweat glands to differentiate the two sweat glands.

Introduction

Apocrine and eccrine sweat glands are two types of sweat glands distributed close to each other in the region of axillary area (Beer et al. 2006). Bromhidrosis has a significant impact on personal occupation and social psychology (Bragança et al. 2014). So far, it is not clear whether bromhidrosis is caused by apocrine sweat glands or the co-action of apocrine sweat glands and eccrine sweat glands. Some studies showed that the characteristic odor of bromhidrosis was caused only by the apocrine glands, as the number of apocrine glands was increased compared to normal people, while the eccrine glands were unchanged (Shinaoka et al. 2021; Beer et al. 2006; Bang et al. 1996). However, some studies considered that the special odor was the result of the co-action of apocrine sweat glands and eccrine sweat glands for that the bromhidrosis had a higher density of eccrine and apocrine sweat glands (Lonsdale-Eccles et al. 2003; Semkova et al. 2015). Therefore, to clarify the issue, we must first find reliable markers to distinguish between apocrine sweat glands and eccrine sweat glands.

Apocrine sweat glands and eccrine sweat glands are different in the distribution, structure and function (Lu and Fuchs 2014; Sato et al. 1989; Borowczyk-Michalowska et al. 2017; Lin et al. 2021). In humans, apocrine sweat glands are mainly distributed in the axillae, ear canal, breast, eyelid, inguinal and perineal, and are associated with hair follicles (Fuchs 2016; Lu and Fuchs 2014; Lonsdale-Eccles et al. 2003). Apocrine sweat glands are present at birth and do not become active until puberty, but little is known about their function in humans. (Fuchs 2016; Lu and Fuchs 2014; Lonsdale-Eccles et al. 2003). In contrast, the eccrine sweat glands are distributed almost all over the body, independence of hair follicles, and are active from birth (Lu and Fuchs 2014; Sato et al. 1989; Lin et al. 2021). The main function of eccrine sweat glands is to regulate body temperature in hot environments or during physical exercise (Lu and Fuchs 2014; Sato et al. 1989; Lin et al. 2021).
Morphologically, the ducts of the two glands are roughly similar and difficult to distinguish, while the secretory coils of the apocrine sweat glands and the eccrine sweat glands are different (Semkova et al. 2015; Wilke et al. 2006; Borowczyk-Michalowska et al. 2017). Generally, the lumens of the apocrine secretory coils are larger, while the lumens of the eccrine secretory coils are smaller (Lu and Fuchs 2014; Sato et al. 1989; Lin et al. 2021). Apocrine secretory coils have variable appearances in tissue sections, making it sometimes difficult to distinguish apocrine and eccrine sweat glands. In this study, we detected the reported apocrine and eccrine sweat gland markers K7, K18, K19, Na+-K+-2Cl- cotransporter 1 (NKCC1), carbonic anhydrase II (CAII), Forkhead transcription factor a1 (Foxa1), homeobox transcription factor engrailed homeobox1 (En1), gross cystic disease fluid protein-15 (GCDFP-15), mucin-1 (MUC-1), cluster of differentiation 15 (CD15) and apolipoprotein (APOD) by immunofluorescence to find specific markers to differentiate apocrine sweat glands from eccrine sweat glands.

**Materials And Methods**

**Skin samples**

Eight finger skin samples and eight axillary skin samples were used. Finger skin samples were obtained from 3- to 26-year-old individuals undergoing polydactylectomy, and axillary skin samples were obtained from 3- to 30-year-old individuals undergoing axillary surgery unrelated to axillary apocrine glands in Department of Wound Repair and Dermatologic Surgery, Taihe Hospital, Hubei University of Medicine. Ethical permission was granted by the Ethics Committee of Hubei University of Medicine, and informed consents were obtained from patients or their guardians. The samples were fixed in 4% paraformaldehyde, paraffin-embedded and cut into 5 µm-thick sections.

**Hematoxylin and eosin (H&E) staining**

The sections were dewaxed in xylene, rehydrated through grades of alcohol (100%, 95%, 85% and 75%) to double distilled water, stained in H&E and mounted in resin.

**Immunofluorescence staining**

The sections were routinely deparaffinized and hydrated, and then the sections were heated to 95 ◦C in Tris-EDTA buffer (pH 9.0) for 15 min for antigen retrieval. Subsequently, the sections were incubated with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 30 min at 37°C to block nonspecific sites. Third, the sections were incubated respectively with different primary antibodies (data on the primary antibodies are described in Table 1) at 4°C overnight, followed by incubation with Cy3-labeled goat anti-rabbit IgG (H + L) (A0516, Beyotime, Jiangsu, China) for rabbit-derived primary antibodies, Cy3-labeled goat anti-mouse IgG (H + L) (A0521, Beyotime, Jiangsu, China) for mouse-derived primary antibodies, or Cy3-labeled donkey anti-goat IgG (H + L) (A0502, Beyotime, Jiangsu, China) for goat-derived primary antibody, at room temperature in the dark at 1:500 dilution for 1 h. Finally, the
sections were counterstained with 4′, 6-diamidino-2-phenylindole (DAPI; Beyotime, Jiangsu, China) for 2 min at room temperature in the dark and mounted with anti-fade mounting medium (P0128, Beyotime, Jiangsu, China). PBS was used for rinsing between steps. Sections omitting the primary antibodies or using normal serum of the same species as the primary antibodies were used as negative controls.

Table 1
Summary of Primary Antibodies.

<table>
<thead>
<tr>
<th>Primary antibodies</th>
<th>Source</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse anti- CD15 (ab241552)</td>
<td>Abcam, UK</td>
<td>1:200</td>
</tr>
<tr>
<td>Rabbit anti- APOD (Ab108191)</td>
<td>Abcam, UK</td>
<td>1:200</td>
</tr>
<tr>
<td>Mouse anti- CAII (sc-133111)</td>
<td>Santa cruze, USA</td>
<td>1:50</td>
</tr>
<tr>
<td>Goat anti- NKCC1 (sc-21545)</td>
<td>Santa cruze, USA</td>
<td>1:200</td>
</tr>
<tr>
<td>Rabbit anti- En1 (HPA073141)</td>
<td>Atlas, Sweden</td>
<td>1:50</td>
</tr>
<tr>
<td>Rabbit anti- MUC-1 (ab109185)</td>
<td>Abcam, UK</td>
<td>1:250</td>
</tr>
<tr>
<td>Rabbit anti- GCDFP-15 (ab133290)</td>
<td>Abcam, UK</td>
<td>1:200</td>
</tr>
<tr>
<td>Rabbit anti- K7 (ab181598)</td>
<td>Abcam, UK</td>
<td>1:1000</td>
</tr>
<tr>
<td>Rabbit anti- K18 (ab181597)</td>
<td>Abcam, UK</td>
<td>1:500</td>
</tr>
<tr>
<td>Rabbit anti- K19 (Ab52625)</td>
<td>Abcam, UK</td>
<td>1:500</td>
</tr>
<tr>
<td>Rabbit anti- Foxa1 (ab170933)</td>
<td>Abcam, UK</td>
<td>1:1000</td>
</tr>
</tbody>
</table>

Abbreviations: APOD, apolipoprotein D; CAII, carbonic anhydrase II; NKCC1, Na⁺-K⁺-2Cl⁻ cotransporter 1, EN1, Homeobox transcriptional factor engrailed homeobox1; GCDFP-15, gross cystic disease fluid protein-15; Foxa1, Forkhead transcription factor A1.

Results

Typical eccrine sweat glands could be easily differentiated from apocrine sweat glands morphologically

In axillary skin, apocrine sweat glands and eccrine sweat glands were both located in the dermis of the axillary skin (Fig. 1A). The lumens of apocrine secretory coils (Fig. 1A, A1) were obviously larger than those of eccrine secretory coils (Fig. 1A, A2). In fingers, there were only eccrine sweat glands, and most of the eccrine sweat glands were located in the deep part of the dermis and the upper part of the subcutaneous tissue (Fig. 1B). The morphology of the eccrine sweat glands in the finger skin was similar to that in the axillary skin, with a small lumen (Fig. 1A, 1B).
CD15 and APOD were specific markers of apocrine sweat glands

The expression and localization of all detected antigens were consistent in all skin samples. Negative controls did not show non-specific binding and false positive results (Fig. 2C1-3). CD15 was positive in the apocrine sweat glands (Fig. 2A1; Table 2), while was negative in the eccrine sweat glands (Fig. 2A2, 2A3; Table 2). APOD showed a strong reaction in the apocrine sweat glands (Fig. 2B1; Table 2), but showed an absence of staining in the eccrine sweat glands (Fig. 2B2, 2B3; Table 2).

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Apocrine secretory coins</th>
<th>Eccrine secretory coins</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD15</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>APOD</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CAII</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>NKCC1</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>En1</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>MUC-1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>GCDFP-15</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>K7</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>K18</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>K19</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Foxa1</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Abbreviations: +, expressed; −, not expressed

CAII, NKCC1 and En1 were specific markers of eccrine sweat glands

CAII (Fig. 3A1-3) and NKCC1 (Fig. 3B1-3) stained the cell membrane and cytoplasm, and En1 (Fig. 3C1-3) stained the nucleus. CAII was strongly expressed in the eccrine sweat glands (Fig. 3A2, 3A3; Table 2), but was not expressed in the apocrine sweat glands (Fig. 3A1; Table 2). NKCC1 showed almost no staining in the apocrine sweat glands (Fig. 3B1; Table 2), while showed positive staining in the eccrine sweat glands (Fig. 3B2, 3B3; Table 2). En1 immunoreactivity (Fig. 3C1-3; Table 2) was found in the eccrine sweat glands (Fig. 3C2, 3C3; Table 2), but not in the apocrine sweat glands (Fig. 3C1; Table 2).
MUC-1, GCDFP-15, K7, K18, K19 and Foxa1 were the common antigens of apocrine and eccrine sweat glands.

MUC-1 (Fig. 4A1-3) was expressed in cell membrane, GCDFP-15 (Fig. 4B1-3), K7 (Fig. 4C1-3), K18 (Fig. 4D1-3) and K19 (Fig. 4E1-3) were expressed in cytoplasm, and Foxa1 (Fig. 4F1-3) were expressed in nucleus. Both apocrine sweat glands (Fig. 4A1-F1) and eccrine sweat glands (Fig. 4A2-F2, 4A3-F3) were labelled by MUC-1 (Fig. 4A1-3; Table 2), GCDFP-15 (Fig. 4B1-3; Table 2), K7 (Fig. 4C1-3; Table 2), K18 (Fig. 4D1-3; Table 2), K19 (Fig. 4E1-3; Table 2) and Foxa1 (Fig. 2F1-3; Table 2).

Discussion

Some previous studies have reported the differential antigens between eccrine sweat glands and apocrine sweat glands, but most of these antigens are not true differential antigens, so they cannot be used to distinguish between eccrine sweat glands and apocrine sweat glands. In this study, the expression of a series of antigens in finger skin and axillary skin were detect by immunofluorescence staining to nd specic markers that can differentiate eccrine sweat glands from apocrine sweat glands.

Keratins are the major structural proteins of the epidermis and provide specific markers to differentiate different epithelial cell types (Wang et al. 2016; Fuchs 1995). K7, K18, and K19 are reported to be specific markers for eccrine sweat glands, so we examined their expression in apocrine and eccrine sweat glands (Cao et al. 2019; Hu et al. 2019; Lu et al. 2016; Li et al. 2009; Cui and Schlessinger 2015). The results showed that K7, K18 and K19 were positively expressed in eccrine sweat glands and apocrine sweat glands. Therefore, K7, K18 and K19 cannot be used to as specific markers to distinguish between apocrine and eccrine sweat glands. Likewise, Foxa1 had previously been shown to be expressed in the secretory coils of eccrine sweat glands and involved in eccrine sweat gland development (Li et al. 2017b; Cui et al. 2012). The immunofluorescence staining in our study shows that Foxa1 was negatively expressed in epidermis, hair follicles and sebaceous glands (data not shown), and positively expressed in the secretory coils of apocrine and eccrine sweat glands. Therefore, Foxa1 can be used a specific marker for skin sweat glands, but it is not the desired makers for distinguishing eccrine sweat glands and apocrine sweat glands.

GCDFP-15, also known as BRST-2, is considered a marker of apocrine differentiation (Darrow E. Haagensen et al. 1990; Miura et al. 2018). However, some studies had shown that GCDFP-15 expression was also present in eccrine sweat glands, lacrimal glands, ceruminous glands, Moll's glands, submandibular glands, sublingual glands, salivary glands and bronchial glands (Viacava et al. 1998; Saga 2001). In our study, immunofluorescence staining showed that GCDFP-15 was expressed in both apocrine and eccrine sweat glands. Combined with previous studies, GCDFP-15 may be more suitable as a marker for glandular differentiation, rather than a specific marker for distinguishing apocrine and eccrine sweat glands. MUC1 is constitutively expressed in the epithelial cells (Viragh et al. 2006). It was previously considered to be an apocrine marker that distinguishes apocrine sweat glands from eccrine sweat glands, because human milk fat globule membranes-1 (HMFG-1), a monoclonal antibody detecting
the fully glycosylated MUC1, stained only apocrine sweat glands but not eccrine sweat glands (Viragh et al. 2006). However, in our study, MUC1 was positively expressed not only in apocrine sweat glands, but also in secretory sweat glands. Therefore, MUC-1 is not the specific marker that we are looking for to differentiate between apocrine and eccrine sweat glands.

CAII is a cytoplasmic enzyme that catalyzes the reversible conversion of carbon dioxide and water into carbonic acid, protons and bicarbonate ions, and NKCC1 is an ion transport protein that transport Na, K, and Cl ions inside or outside the cells (Haas and Forbush 2000; Hassan et al. 2013). CAII had been used as a specific marker for clear secretory cells of eccrine sweat glands, and NKCC1 had been used as a specific marker for eccrine sweat glands to distinguish eccrine sweat glands from hair follicles (Cao et al. 2019; Li et al. 2017a). In our study, CAII and NKCC1 were expressed in eccrine sweat glands, but not in the apocrine sweat glands, which was consistent with the study by Bovell et al (Bovell et al. 2011). En1, a member of the homeobox transcription factor family, is a marker of eccrine sweat glands and is involved in the development of eccrine sweat glands (Miura et al. 2018; Kamberov et al. 2015). Ectopic expression of En1 in the hair follicle placodes promoted the differentiation of hair follicle into eccrine sweat glands (Lu et al. 2016). En1 heterozygote mutant mice showed less eccrine sweat glands (Kamberov et al. 2015). A previous study by Miura et al. showed that En1 was specifically expressed in normal eccrine sweat glands and eccrine differentiated tumors, but not in apocrine sweat glands, sebaceous glands and hair follicles (Miura et al. 2018). In our study, the positive nuclear staining for En1 was found in eccrine sweat glands, but negative in apocrine sweat glands, which was consistent with the study by Miura et al. (Miura et al. 2018). Therefore, En1, NKCC1 and CAII can be used as specific markers for eccrine sweat glands to distinguish eccrine sweat glands from apocrine sweat glands.

The glycan determinant CD15, also known as stage specific embryonic antigen 1 (SSEA-1), have originally identified as a monocyte cell marker (Gadhoun and Sackstein 2008; Pellegrini et al. 2007). Previous studies had shown that CD15 is only expressed in apocrine sweat glands but not in the eccrine sweat glands (Wilke et al. 2006; Bovell et al. 2011; Ansai et al. 1995; Bovell et al. 2007). APOD is an extracellular glycoprotein of the lipocalin family involved in lipid transport, food intake and development (Rassart et al. 2020). A previous study showed that APOD was expressed in the apocrine sweat glands of patients with bromhidrosis and healthy subjects, but the expression level of APOD in patients with bromhidrosis was twice that of healthy subjects (Chen et al. 2013). In our study, CD15 and APOD were only expressed in the apocrine sweat glands. Therefore, CD15 and APOD can be used as specific markers for apocrine sweat glands to distinguish apocrine sweat glands from eccrine sweat glands.

In conclusion, CD15 and APOD can be used as specific markers for apocrine sweat glands, whereas NKCC1, CAII and En1 can be as specific markers for eccrine sweat glands to distinguish between eccrine and apocrine sweat glands.

Declarations

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Competing interest's statement

We declare we have no competing financial, personal or other relationships with other people or organizations.

References


Figures

Figure 1

The morphology and distribution of eccrine sweat glands and apocrine sweat glands in axillary skin and finger skin.

Eccrine sweat glands and apocrine sweat glands in the axillary skin in H&E staining (A, A1, A2). A higher magnification of apocrine sweat glands (A1) and eccrine sweat glands (A2) in the boxed areas in A. Eccrine sweat glands in the finger skin in H&E staining (B, B1). A higher magnification of eccrine sweat glands in the boxed areas in B (B1). The scale bars are 50 μm.
**Figure 2**

**Specific markers for apocrine sweat glands.**

Immunofluorescence staining of CD15 (A1-3), APOD(B1-3) and Negative control (C1-3) in axillary apocrine sweat glands (A1-C1), axillary eccrine sweat glands (A2-C2), and finger eccrine sweat glands (A3-C3). The scale bar is 50 μm. Abbreviations: APOD, apolipoprotein D; NC, Negative control.
Figure 3

Specific markers for eccrine sweat glands.

Immunofluorescence staining of CAII (A1-3), NKCC1 (B1-3) and En1 (C1-3) in axillary apocrine sweat glands (A1-C1), axillary eccrine sweat glands (A2-C2), and finger eccrine sweat glands (A3-C3). The scale bar is 50 μm. Abbreviations: CAII, carbonic anhydrase II; NKCC1, Na+-K+-2Cl- cotransporter 1; En1, engrailed 1.
Figure 4

Common markers of apocrine sweat glands and eccrine sweat glands.

Immunofluorescence staining of MUC-1 (A1-3), GCDFP-15 (B1-3), K7 (C1-3), K18 (D1-3), K19 (E1-3) and Foxa1 (F1-3) in axillary apocrine sweat glands (A1-F1), axillary eccrine sweat glands (A2-F2), and finger
eccrine sweat glands (A3-F3). The scale bar is 50 μm. Abbreviations: GCDFP-15, gross cystic disease fluid protein-15; Foxa1, Forkhead transcription factor a1.