Acta Histochemica xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.com/locate/acthis

Expression and localization of Forkhead transcription factor A1 in the three-dimensional reconstructed eccrine sweat glands

Haihong Li[a,b,*,1], Liyun Chen[a,1], Mingjun Zhang[a], Sitian Xie[a], Liuhanghang Cheng[a]

[a] Burn and Plastic Surgery, The Second Affiliated Hospital, Shantou University Medical College, North Dongxia Road, Shantou, 515041, Guangdong Province, China
[b] Research Center for Translational Medicine, Shantou University Medical College, North Dongxia Road, Shantou, 515041, Guangdong Province, China

ARTICLE INFO

Keywords:
Eccrine sweat glands
Three-dimensional reconstruction
FoxA1
GCDFP-15
Na^+-K^+-ATPase α
NKCC1

ABSTRACT

Previously studies showed that Forkhead transcription factor A1 (FoxA1) was associated with sweat secretion. To investigate the expression and localization of FoxA1 in the three-dimensional (3D) reconstructed eccrine sweat glands, eccrine sweat gland cells were transplanted subcutaneously into nude mice with Matrigel, and at 2, 3, 4, 5, 6, 8, 10 and 12 weeks post-transplantation, the reconstructed eccrine sweat glands were removed and immunostained for FoxA1 and co-immunostained for FoxA1 and eccrine sweat markers, K7, carbonic anhydrase II (CA II), gross cystic disease fluid protein-15 (GCDFP-15) and α-smooth muscle actin (α-SMA), and FoxA1 and sweat secretion-related proteins, Na^+-K^+-ATPase α and Na^+-K^+-2Cl- cotransporter 1 (NKCC1). The results showed that FoxA1-positive cells weren't detected until 3 weeks post-implantation, a time point of the differntiation of secretory coil-like structures. From the fourth week on, the number of FoxA1-positive cells increased and thereafter maintained at a high number. Double immunofluorescence staining showed that FoxA1-positive cells co-expressed dark cell marker GCDFP-15 and myoepithelial cell marker α-SMA, as well as secretion-related proteins, Na^+-K^+-ATPase α and NKCC1 in both the native and reconstructed eccrine sweat glands. In conclusion, FoxA1 might be related to the development and differentiation of secretory coil-like structures, as well as the secretory function of the 3D reconstructed eccrine sweat glands.

1. Introduction

Eccrine sweat gland is a simple single tubular structure composed of a duct and a secretory coil (Cui and Schlessinger, 2015; Saga, 2002; Sato et al., 1989). The duct is straight and opens directly onto the skin surface for sweat fluids excretion (Cui and Schlessinger, 2015; Saga, 2002; Sato et al., 1989). The secretory coil contains three types of cells, namely dark secretory cells, clear secretory cells and myoepithelial cells (Cui and Schlessinger, 2015; Saga, 2002; Sato et al., 1989). Myoepithelial cells surround secretory cells, which provide contractile support to facilitate sweat secretion (Cui and Schlessinger, 2015; Saga, 2002; Sato et al., 1989, 1979). Dark secretory cells and clear secretory cells are both responsible for producing sweat and its ingredients, with clear secretory cells mainly secreting water, electrolytes and inorganic substances, and dark secretory cells secreting macromolecules such as glycoproteins (Cui and Schlessinger, 2015; Saga, 2002; Sato et al., 1989). Dark and clear secretory cells co-express K7, K8, K15, K18 and K19, with clear secretory cells specifically expressing carbonic anhydrase II (CA II) and dark secretory cells specifically expressing gross cystic disease fluid protein-15 (GCDFP-15) (Bovell et al., 2011; Li et al., 2017a, 2009; Saga, 2001, 2002). Myoepithelial cells are positive for K5, K14, K17 and α-SMA (Li et al., 2015b; Moll and Moll, 1991, 1992; Saga, 2002). The primary function of eccrine sweat glands is sweat secretion, which is important in the regulation of human body temperature (Saga, 2002; Sato et al., 1989). Na^+-K^+-2Cl- co-transport model is recognized as the main mechanism of sweat secretion, and Na^+-K^+-ATPase, Na^+-K^+-2Cl- cotransporter (NKCC), K^+ channel and Cl- channel are the common features of the model (O'Grady et al., 1987; Saga, 2002).

Forkhead transcription factor A1 (FoxA1) played critical roles in multiple processes of mammalian life (Friedman and Kaestner, 2006). It enabled chromatin combined with tissues specific transcription factors through binding to promoters and enhancers (Friedman and Kaestner, 2006). Skin-specific FoxA1-deficient mice showed complete eccrine sweat gland structures but absolute anhidrosis (Cui et al., 2012). Further study demonstrated that FoxA1 was involved in the secretory

Abbreviations: 3D, three-dimensional; α-SMA, α-smooth muscle actin; Best2, Bestrophin 2; CA II, carbonic anhydrase II; DAPI, 4', 6-diamidino-2-phenylindole; FoxA1, Forkhead transcription factor A1; GCDFP-15, gross cystic disease fluid protein-15; NKCC, Na^+-K^+-2Cl-cotranspoter; PBS, phosphate-buffered saline; SUMC, Shantou University Medical College

* Corresponding author.
E-mail address: lihaihong1051@126.com (H. Li).
[1] Haihong Li and Liyun Chen are co-first authors.

https://doi.org/10.1016/j.acthis.2018.06.003
Received 14 February 2018; Received in revised form 24 May 2018; Accepted 11 June 2018

function of eccrine sweat glands through FoxA1-Ca^{2+}-activated anion channel Bestrophin 2 (Best2) cascade and FoxA1-Na^+-K^+-2Cl- co-transporter 1 (NKCC1) cascade (Cui et al., 2012). Best2 and NKCC1 were the two key targets of sweat secretion, so FoxA1 was a master regulator of sweating (Cui et al., 2012; Saga, 2002).

The development of human eccrine sweat glands is complete before birth, and they cannot regenerate after birth (Cui and Schlessinger, 2015; Lu et al., 2012; Saga, 2002). For patients suffered severe second-degree or third-degree burns, most of their eccrine sweat glands were lost or destroyed, which led the patients to be hyperthermia and even heat stroke, so repair and regeneration of the damaged or lost eccrine sweat glands are becoming urgent needs (Castleberry et al., 2016; Fu et al., 2005; Pearson et al., 2017). We previously had established a three-dimensional (3D) reconstructed model of eccrine sweat glands and demonstrated that the reconstructed eccrine sweat glands had similar structures as those of native ones, with lumens and intact basement membrane, expression of sweat gland markers and differentiation into secretory- and ductal-like structures (Li et al., 2015a, 2016; Li et al., 2017c, d). However, whether the 3D reconstructed glands possessed function as the native ones did was still unclear. In the study, we examined the expression and localization of FoxA1, one of the master regulators of sweating in native eccrine sweat glands, in 3D reconstructed eccrine sweat glands.

2. Materials and methods

2.1. Primary culture of eccrine sweat gland cells

Non-cauterized and 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 (SUMC). The patients' mean age was 20 ± 2 years and the regions of specimens included fingers, abdomens, arms, and legs. Ethical permission was granted by the Ethics Committee of SUMC (Shantou, China), and informed consent was obtained from patients or their guardians. A small portion of the specimens was fixed in 4% paraformaldehyde, paraffin-embedded and stained for immunofluorescence. The rest of the specimens was processed for culture of eccrine sweat gland cells

Human eccrine sweat glands were isolated as previously described (Li et al., 2015a, 2016). Briefly, skin specimens were minced and digested with 2 mg/ml collagenase type II (Gibco, USA), and the free eccrine sweat gland tissues were picked up and cultured in defined keratinocyte serum-free medium (Gibco, USA). When reached 70% confluence, the cells were harvested for the cell implantation experiments.

2.2. Cell implantation experiments

Cell implantation was performed as previously described (Li et al., 2015a, 2016). In short, twenty-four normal NU/NU athymic mice were obtained from the Vital River Laboratory Animal Technology Company Limited (Beijing, China). After the mice were anesthetized, the mixtures of 3 million eccrine sweat gland cells and 500 µl Matrigel (BD Bioscience, USA) was injected subcutaneously into the inguinal region of each mouse. At 2, 3, 4, 5, 6, 8, 10 and 12 weeks post-implantation, three mice were euthanized at each time point and Matrigel plugs were removed, fixed, embedded and stained for immunofluorescence.

2.3. Immunofluorescence staining for FoxA1

Immunofluorescence staining was performed as previously described (Li et al., 2017b). Briefly speaking, the sections were deparaffinized and rehydrated routinely, and were heated to 95 °C in 0.01 M citric acid buffer (pH 6.0) for 15 min for antigen retrieval. Subsequently, the sections were incubated with rabbit anti-FoxA1 (1:100 dilution, ab170933, Abcam, Cambridge, UK), followed by incubation with Cy3-labeled goat anti-rabbit IgG (H + L) secondary antibody (1:500 dilution, A0516, Beyotime, Shanghai, China) and counterstained with 4', 6-diamidino-2-phenylindole (DAPI, C1005, Beyotime, Shanghai, China). PBS was used for rinsing between steps. Sections omitting the primary antibodies were used as negative controls. The staining results were observed under fluorescence microscopy (Olympus, Japan). The percentage of positive cells = (the number of positive cells/total number of cells) × 100%. The values were expressed as mean.

2.4. Double immunofluorescence staining for FoxA1 and cell type markers, as well as FoxA1 and sweat secretion-related proteins

The protocol for double immunofluorescence staining was similar to the immunofluorescence staining procedure except for the following steps. For sections stained for rabbit anti-FoxA1/mouse anti-α-SMA (BM0002, Boster, Wuhan, China), and rabbit anti-FoxA1/mouse anti-Na^+-K^+-ATPase α (sc-58628; Santa Cruz, USA), the sections were incubated with primary antibodies together, and then with the secondary antibodies, Alexa Fluor 488-labeled goat anti-mouse IgG (1:500 dilution, A0428, Beyotime, Shanghai, China) and Cy3-labeled goat anti-rabbit IgG, together.

For sections stained for rabbit anti-FoxA1/rabbit anti-K7 (ab181598, Abcam, Cambridge, UK), rabbit anti-FoxA1/rabbit anti-CA II (ab124687, Abcam, Cambridge, UK), rabbit anti-FoxA1/rabbit anti-GCDFP-15 (ab133290, Abcam, Cambridge, UK) and rabbit anti-FoxA1/rabbit anti-NKCC1 (sc-21545; Santa Cruz, USA), the sections were first incubated with rabbit anti-FoxA1, followed by incubation with Cy3-labeled goat anti-rabbit IgG (H + L) secondary antibody. Subsequently, the sections were incubated with others rabbit anti- primary antibodies respectively for 10 min, followed by incubation with Alexa Fluor 488-labeled goat anti-rabbit IgG (H + L) secondary antibody (A0423, Beyotime, Shanghai, China) for 5 min in the dark.

3. Results

3.1. FoxA1 was first detected in 3D reconstituted human eccrine sweat glands at 3 weeks post-implantation

There was no positive staining in all the negative control (Fig.1I). FoxA1, expressing in the nuclei, was undetectable at 2 weeks post-implantation (Fig.1A; Fig.2). At 3 weeks post-implantation, the expression of FoxA1 was first detected (Fig.1B; Fig.2). At 3 and 4 weeks, the number of FoxA1 positive cells was few (Fig.1B,C; Fig.2), but from 5 weeks on, FoxA1 positive cells increased apparently, and thereafter maintained at a high positive expression (Fig.1D–H; Fig.2).

3.2. FoxA1 was mainly expressed in the dark cells and a few in myoepithelial cells in both the 3D reconstructed and native eccrine sweat glands

To detect which kind of cells expressed FoxA1, the sections were doubly stained for anti-FoxA1 antibody and anti-K7 antibody, one of the markers of secretory coils. The staining results showed FoxA1-positive cells were present exclusively in K7-positive structures (Fig. 3A,E; arrows), indicating FoxA1-positive cells were localized in secretory coils in both natives (Fig. 3E) and 3D reconstructed (Fig. 3A) eccrine sweat glands. Second, FoxA1 was co-immunostained with dark secretory marker GCDFP-15, clear secretory marker CA II and myoepithelial cell marker α-SMA to differentiate which types of FoxA1-positive cells were belonging to. The results showed that FoxA1-positive cells co-expressed GCDFP-15 (Fig. 3C, 3 G; arrows) and α-SMA (Fig. 3D, H; arrows) in both the native (Fig. 3G, H) and reconstructed (Fig. 3C, D) eccrine sweat glands, but FoxA1-positive cells did not co-express CA II (Fig. 3B, F; asterisks), which suggested that FoxA1-positive cells were