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BrdU-label-retaining cells in rat eccrine sweat glands over time

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ABSTRACT

Cell proliferation and turnover are fueled by stem cells. In a previous study, we demonstrated that rat eccrine sweat glands contained abundant bromodeoxyuridine (BrdU)-label-retaining cells (LRCs). However, morphological observations showed that eccrine sweat glands usually show little or no signs of homeostatic change. In this study, we account for why the homeostatic change is rare in eccrine sweat glands based on cytokinetic changes in BrdU-LRC turnover, and also determine the BrdU-labeled cell type. Thirty-six newborn SD rats, were injected intraperitoneally with 50 mg/kg BrdU twice daily at a 2 h interval for 4 consecutive days. After a chase period of 4, 6, 8, 12, 24 and 32 weeks, rats were euthanized, and the hind footpads were removed and processed for BrdU immunostaining, and BrdU/α-SMA and BrdU/K14 double-immunostaining. BrdU-LRCs were observed in the ducts, secretory coils and mesenchymal cells at all survival time points. The percentage of $BrdU^+$ cells in rat eccrine sweat glands averaged $4.2 \pm 1.2\%$ after 4 weeks of chase, increased slightly by the 6th week, averaging $4.4 \pm 0.9\%$, and peaked at 8 weeks, averaging $5.3 \pm 1.0\%$. Subsequently, the average percentage of $BrdU^+$ cells declined to $3.2 \pm 0.8\%$ by the 32nd week. There was no difference in the percentage of BrdU-LRCs among the different survival time points except that a significant difference in the percentage of BrdU-LRCs detected at 24 weeks *versus* 8 weeks, and 32 weeks *versus* 8 weeks, was observed. We concluded that the BrdU-LRCs turnover is slow in eccrine sweat glands.

Introduction

Eccrine sweat glands are the most abundant glandular epithelium of the human body, and are essential for controlling body temperature, allowing humans to live in diverse climates (Rittie et al., 2013; Saga, 2002). Eccrine sweat glands are composed of a secretory portion and duct. The secretory portion of eccrine sweat gland is pseudostratified epithelium, comprised of secretory cells and myoepithelial cells. Myoepithelial cells surround the secretory cells, and basement membranes surround both myoepithelial cells and secretory cells (Saga, 2002). The duct is a stratified epithelium, that is composed of two layers of cells: the peripheral ductal cells and the luminal cells (Saga, 2002).

Stem cells are multipotent, slow cycling and self-renewing cells (Ma et al., 2004). In the absence of, or up to two cell divisions, stem cells can retain a nucleotide-labeled DNA in the nucleus over several weeks, or even several months. The label-retaining cells (LRCs) have been identified as the tissue stem cells (Ma et al., 2004). Multiple tissues, such as epidermis and hair follicles, are known to harbour stem cells, as do the eccrine sweat glands (Chen et al., 2014; Kim et al., 2004; Lu et al., 2012; Ma et al., 2004; Rahmani et al., 2014).

Cell proliferation and turnover are fueled by stem cells (Lu et al., 2012). In previous studies, we and others demonstrated that rat eccrine sweat glands contain abundant BrdU-LRCs (Chen et al., 2014; Lu et al., 2012). However, morphological observations showed that eccrine sweat glands usually show little or no signs of homeostatic change (Lu et al., 2012). In this study, from cytokinetic changes and stem cell biology, we account for the discrepancy between the abundant stem cells and the rare homeostatic change in eccrine sweat glands. We administered BrdU to neonatal rats and, at various times after BrdU injection, determined both the

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percentage of BrdU-LRCs in eccrine sweat glands and the cell types labeled with BrdU. We conclude that BrdU-LRCs turnover is slow in eccrine sweat glands, which may partially account for why the eccrine sweat glands usually show little or no signs of homeostatic change.

Materials and methods

Animal experiments

Virgin female and male Sprague–Dawley (SD) rats, weighing 180–200 g, were purchased from the Laboratory Animal Center of Shantou University Medical College (Shantou, China) and maintained on a 12-h light/dark cycle with food and tap water *ad libitum*. Room temperature was maintained at 22 ± 1 °C with low humidity. One week later, females were caged with males (ratio 4:1) at night. Pregnancy was confirmed by a sperm-positive vaginal smear. Once the female rats were demonstrably pregnant, they were caged separately. The animals and procedures used in this study were in accordance with the guidelines and approval of the Shantou University Medical College Animal Care and Use Committee.

To label stem cells in rat eccrine sweat glands, we used the peculiar very slow cycling characteristic of stem cells that allows them to retain nucleotide-labeled DNA in the nucleus for over several weeks or months. We performed BrdU pulse-chase experiments in neonatal SD rats. Thirty-six newborn rats within 24 h after birth were intraperitoneally injected with 50 mg/kg BrdU twice daily at a 2 h interval for 4 consecutive days. Rats were euthanized 4, 6, 8, 12, 24 and 32 weeks after the last BrdU injection, and then were perfused with ice-cold 0.1 M PBS and 4% paraformaldehyde. Hind footpads were removed, fixed in 4% paraformaldehyde, dehydrated, and embedded in paraffin. Embedded hind footpads were sectioned and stained.

Immunohistochemical analysis of LRCs

Five micrometer-thick tissue sections were deparaffinized and endogenous peroxidase was blocked by immersing sections in 3% H_2O_2 in methanol for 5 min. Antigen retrieval was performed by heating the sections to 95 °C in a citric acid buffer (pH 6.0) for 15 min and slowly cooling to room temperature. Nonspecific binding sites were blocked by incubation in PBS containing 10% normal goat serum and the slides were then incubated with mouse anti-BrdU antibody (Chemicon, USA, MAB4072) at 1:1000 dilution for 24 h at 4 °C, followed by incubation with secondary horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (Beyotime, China, A0216) for 1 h at room temperature at 1:100 dilution. Finally, sections were developed in 3, 3′-diaminobenzidine (DAB) (ZGSB-BIO, China, ZLI-9018), counterstained with haematoxylin and mounted in neutral resin. PBS was used for rinsing between steps. Sections omitting the primary antibodies were used as negative controls. Each animal was represented by two hind footpads. Three sections, at least 50 μm apart from each footpad were evaluated. Five randomly selected microscope fields (400×) were observed in each section. Both the $BrdU^+$ and $BrdU^-$ sweat gland cells in each section were counted manually and then the percentage of $BrdU^+$ cells in the eccrine sweat glands at various survival time points was calculated. The percentage of $BrdU^+$ cells in the eccrine sweat glands = $BrdU^+$ cells/($BrdU^+$ cells + $BrdU^-$ cells) × 100%. All sections were observed under an Olympus BX51 microscope (Tokyo, Japan).

BrdU/α-SMA and BrdU/K14 double immunostaining

Antigen-unmasked sections, prepared as above, were stained for BrdU/α-SMA or BrdU/K14 using the DouSP^{TM} double-staining kit according to the instructions provided by the manufacturer (MXB, China, KIT-9999). Briefly, slides were incubated with mouse anti-BrdU antibody (Chemicon, USA) at a dilution of 1:1000 overnight at 4 °C, and then were incubated with biotinylated second antibody, then incubated with streptavidin alkaline phosphatase for 10 min at room temperature. Sections were developed in 5-bromo-4-chloro-3-indolyl phosphate (BCIP)/nitro blue tetrazolium (NBT) solution for 10 min, and positive BrdU staining was indicated by blue-black nuclei. Subsequently, the sections were incubated with mouse anti-α-SMA primary antibody (BOSTER, China, BM0002) or rabbit anti-K14 primary antibody (ZSGB-BIO, China, ZA0540) at a dilution of 1:100 overnight at 4 °C, respectively, and then were incubated with biotinylated secondary antibody and streptavidin peroxidase for 10 min at room temperature, respectively. Sections were developed in 3-amino-9-ethylcarbazole (AEC) solution for 10 min, to show positive α-SMA or K14 staining as red. Finally, sections were counterstained with haematoxylin and mounted in clear mount. PBS was used for rinsing between steps. Sections omitting the primary antibodies were used as negative controls. All sections were observed under the Olympus BX51 microscope (Tokyo, Japan).

Statistical analysis

Data were analyzed using standard statistical software (SPSS 10.0, Chicago, IL, USA). The values were expressed as mean ± standard deviation. The statistical significance of values among groups was evaluated by one way analysis of variance (ANOVA), followed by Fisher's least significant difference (LSD) post-hoc test. The difference was considered significant when the p value was 0.05 or less.

Results

Percentage of BrdU-LRCs in rat eccrine sweat glands at various times after BrdU administration

Following the 4- (Fig. 1A), 6- (Fig. 1B), 8- (Fig. 1C), 12- (Fig. 1D), 24- (Fig. 1E), and 32-week (Fig. 1F) chase periods, the number and distribution of $BrdU^+$ cells were determined in the rat eccrine sweat glands. $BrdU^+$ cells in the eccrine glands were distributed in a scattered pattern (Fig. 1). At all the observed survival times, both solid BrdU staining (Fig. 1A–F, arrows) and speckled BrdU staining (Fig. 1A–F, arrowheads) could be seen (Fig. 1). BrdU immunoreactivity was also associated with the eccrine sweat gland mesenchymal cells (Fig. 1A–F, asterisks) and cells in the basal layer of the epidermis (Fig. 1G, arrows). No $BrdU^+$ cells were present in the intraepidermal duct (Fig. 1H).

The percentage of $BrdU^+$ cells in rat eccrine sweat glands averaged 4.2 ± 1.2% at 4 weeks after the last BrdU administration, increased slightly by the 6th week, with the average being 4.4 ± 0.9%, and peaked at 8 weeks, averaging 5.3 ± 1.0% (Fig. 4). Subsequently, the average percentage of $BrdU^+$ cells declined to 3.2 ± 0.8% by the 32nd week (Fig. 4). Data analysis using one-way ANOVA showed a statistically significant difference among the 6 time points [$F(5,35) = 2.5853$, $n = 6$, $p < 0.05$]. The Fisher's LSD post-hoc t-test found a statistically significant decline from the 8th to 24th week values ($p < 0.05$), and the 8th to 32nd week values ($p < 0.01$) (Fig. 4).

Cell types of BrdU-LRCs

Double staining of BrdU/α-SMA showed that in the eccrine sweat gland secretory coils, BrdU-labeled myoepithelial cells were $BrdU^+/\alpha\text{-SMA}^+$ (Fig. 2A and B, arrows), whereas BrdU-labeled secretory cells were $BrdU^+/\alpha\text{-SMA}^-$ (Fig. 2A, arrowheads). In the duct (Fig. 2D, double arrows) and the stratum basale of the epidermis (Fig. 2E, double asterisks), BrdU-LRCs were negative for α-SMA