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Original research article

Merkel cells are long-lived cells whose production is stimulated by skin injury☆

Margaret C. Wright[a,*], Gregory J. Logan[b], Alexa M. Bolock[c], Adam C. Kubicki[c], Julie A. Hemphill[c], Timothy A. Sanders[d], Stephen M. Maricich[c,**,1]

[a] Center for Neurosciences, University of Pittsburgh, Pittsburgh, PA 15260, United States
[b] Department of Pathology, University of Pittsburgh, Pittsburgh, PA 15260, United States
[c] Richard King Mellon Institute for Pediatric Research, Department of Pediatrics, University of Pittsburgh and Children's Hospital of Pittsburgh of University of Pittsburgh Medical Center, Pittsburgh, PA 15224, United States
[d] Division of Neonatology, Department of Pediatrics, US Benioff Children's Hospital, University of California San Francisco, San Francisco, CA 94143, United States

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ABSTRACT

Mechanosensitive Merkel cells are thought to have finite lifespans, but controversy surrounds the frequency of their replacement and which precursor cells maintain the population. We found by embryonic EdU administration that Merkel cells undergo terminal cell division in late embryogenesis and survive long into adulthood. We also found that new Merkel cells are produced infrequently during normal skin homeostasis and that their numbers do not change during natural or induced hair cycles. In contrast, live imaging and EdU experiments showed that mild mechanical injury produced by skin shaving dramatically increases Merkel cell production. We confirmed with genetic cell ablation and fate-mapping experiments that new touch dome Merkel cells in adult mice arise from touch dome keratinocytes. Together, these independent lines of evidence show that Merkel cells in adult mice are long-lived, are replaced rarely during normal adult skin homeostasis, and that their production can be induced by repeated shaving. These results have profound implications for understanding sensory neurobiology and human diseases such as Merkel cell carcinoma.

1. Introduction

A primary function of mammalian skin is to provide a protective barrier against environmental insults. Trauma to skin cells necessitates their frequent replacement by resident skin progenitors to maintain skin integrity (Levy et al., 2005; Page et al., 2013). These progenitor cells maintain the barrier function of the skin and insure that skin appendages such as hair follicles, sebaceous and sweat glands continue to function (Jensen et al., 2009; Lu et al., 2012). Skin cell turnover occurs on a regular schedule (for instance, as part of the hair cycle) and as needed following injury (Hsu et al., 2011; Jaks et al., 2008). Different progenitor cell populations located in different regions of the skin participate in these processes (Horsley et al., 2006; Ito et al., 2005; Levy et al., 2005, 2007).

Merkel cells are mechanosensitive cells found in mammalian hairy skin, whisker follicles and glabrous (non-hairy) skin of the hands and feet (Halata et al., 2003). Merkel cells are innervated by slowly-adapting type 1 (SA1) afferent neurons, and these Merkel cell-neurite complexes detect certain light touch stimuli (Iggo and Muir, 1969; Johnson and Hsiao, 1992; Johnson and Lamb, 1981; Maricich et al., 2012, 2009). Reported variations in Merkel cell numbers during the hair cycle (Moll et al., 1996; Nakafusa et al., 2006) and genetic lineage tracing studies (Doucet et al., 2013; Van Keymeulen et al., 2009; Wright et al., 2015; Xiao et al., 2015) suggest that, like other skin cells, adult Merkel cells are regularly replaced. However, the frequency of Merkel cell replacement and identities of Merkel cell progenitors remain unclear.

We analyzed Merkel cell lifespan by EdU birthdating studies beginning in embryogenesis, leading to the unexpected discovery that they persisted into late adulthood. This prompted us to perform a

Abbreviations: DTA, diphtheria toxin subunit A; E, embryonic age; EdU, 5-ethynyl-2′-deoxyuridine; GFP, green fluorescent protein; K8, Keratin 8; MCC, Merkel cell carcinoma; P, postnatal age in days; SA1, slowly adapting type 1 afferent; YFP, yellow fluorescent protein

☆ This work was performed in Pittsburgh, Pennsylvania, USA.

* Correspondence to: 203 Lothrop St, Pittsburgh, PA 15213, United States.
** Correspondence to: 105 Digital Drive, Novato CA 94949, United States.
E-mail addresses: mcw47@pitt.edu (M.C. Wright), stephen.maricich@bmrn.edu (S.M. Maricich).
[1] Current address: BioMarin Pharmaceutical Inc., Novato, CA 94949, United States.

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multifaceted analysis to investigate the kinetics of Merkel cell production, survival and replacement. Surprisingly, we found that touch dome Merkel cell numbers are constant throughout the hair cycle and that new Merkel cells are infrequently generated during adult skin homeostasis. We repeatedly visualized the same touch domes over many months using confocal microscopy in living adult transgenic mice. Consistent with our EdU birthdating studies, we observed that a significant number of Merkel cells lived for longer than 5 months. Furthermore, we illustrate that large numbers of new Merkel cells were generated only in the setting of Merkel cell loss induced by repeated shaving and confirm that these new Merkel cells arise from touch dome keratinocytes. These data reveal important insights into Merkel cell biology that have potential relevance for understanding peripheral somatosensation and the development of Merkel cell carcinoma.

2. Results

2.1. Embryonic Merkel cells persist into late adulthood and new Merkel cells are rarely made during adult skin homeostasis

Keratin (K)14-expressing epidermal progenitors generate the first set of murine Merkel cells in late embryogenesis, beginning at embryonic day (E)14.5 and continuing until birth (Wright et al., 2015). However, it is unclear for how long this initial cohort of Merkel cells survives into postnatal life. To quantify the lifespan of these embryonic-born Merkel cells, we employed a birthdating approach using the modified nucleoside 5-ethynyl-2′-deoxyuridine (EdU). Incorporation of modified nucleosides like EdU into DNA occurs during S-phase; cells that become post-mitotic after incorporation retain EdU throughout their lives, while the signal is diluted until it becomes undetectable (~2 to 5 cell divisions) in cells that continue to divide (Ganusov and De Boer, 2012; Kiel et al., 2007). To determine the lifespan of Merkel cells created during embryogenesis, we administered EdU (10 mg/kg) by once daily intraperitoneal injection to pregnant C57Bl/6 J female mice at E14.5, E15.5, and E16.5, the ages of peak Merkel cell generation (Wright et al., 2015). We harvested and sectioned skin from progeny mice of the same litters at postnatal day (P)0, P21 and 9 months of age (n=4 mice/age), immunostained for the Merkel cell marker Keratin 8 (K8), visualized EdU, then calculated the percentage of K8+ cells that were also EdU+(Fig. 1). Qualitatively, the robustness of the EdU signal in K8+ cells was similar at all three ages, suggesting that the majority of cells labeled during embryogenesis that survived into adulthood did not continue to divide over time. Percentages of K8+EdU+/K8+ cells did not change between P0 and 9 months of age in back and belly skin or in whisker follicles ($p=0.37$ and 0.95, *t*-test; Fig. 1A-D‴, H), nor between P21 and 9 months of age in the glabrous skin of the forepaw (Fig. 1F-G‴, H). A decrease in the forepaw between P0 and P21 was noted, likely secondary to continued production of Merkel cells within the glabrous skin at this age ($p=0.003$, one-way ANOVA, P0 vs. P21 $p < 0.05$, P0 vs. 9 mo. $p < 0.01$, P21 vs. 9 mo. $p > 0.05$; Fig. 1E-F‴, H). These data indicate that Merkel cells born during embryogenesis survive for at least 9 months after becoming post-mitotic.

We were surprised to see persistence of embryonic-born Merkel cells out to 9 months of age, as previous lines of evidence suggested that this cell population should have undergone multiple rounds of complete turnover and replacement during this time (Doucet et al., 2013; Nakafusa et al., 2006; Xiao et al., 2015). We re-examined the frequency of Merkel cell production during the first hair cycle (P21-P56), a five week period of time during which approximately 50% of the Merkel cell population would be predicted to have been generated and incorporated into touch domes (Doucet et al., 2013). EdU was administered in the drinking water (0.2 mg/mL) to P21 C57Bl/6J female mice for 5 weeks, after which time back and belly skin, whisker follicles, and glabrous skin of the forepaw was retrieved and processed for K8 and EdU (Fig. 2A-C‴; n=3 mice). We verified that EdU exposure did not cause Merkel cell loss, as mice that received EdU and age-matched untreated mice had comparable numbers of K8+ cells/touch dome (20.3 ± 2.5 vs 16.7 ± 0.7, respectively, $p=0.41$, *t*-test;

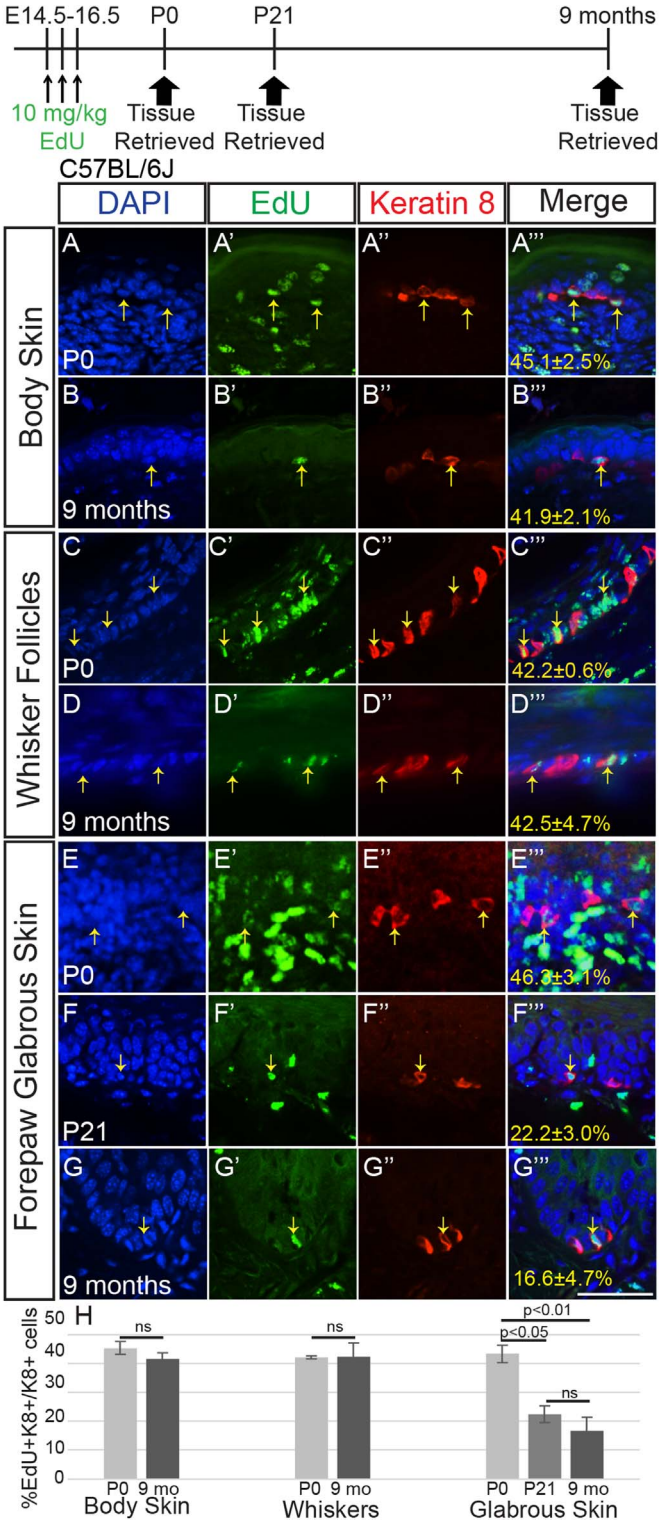

Fig. 1. K8+ cells born in embryogenesis survive at least 9 months. Single z-slice confocal images of sectioned back skin (A-B‴), whisker follicles (C-D‴) and glabrous forepaw skin (E-G‴) from P0 (A-A‴, C-C‴, E-E‴), P21 (F-F‴) and 9 month-old (B-B‴, D-D‴, G-G‴) female C57Bl/6 J mice that received 10 mg/kg EdU at E14.5, 15.5 and 16.5. Tissues were processed for EdU (A'-G', green) and K8 immunostaining (A''-G'', red). Yellow arrows indicate K8+EdU+ cells. Exposure times are similar for all panels. (H) Average percentages (± SEM) of K8+ cells that were EdU+ in each skin region (n=4 mice/age). Scale bar: 50 μm.

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