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Sox2 modulates the function of two distinct cell lineages in mouse skin ☆

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ABSTRACT

In postnatal skin the transcription factor Sox2 is expressed in the dermal papilla (DP) of guard/awl/auchene hair follicles and by mechanosensory Merkel cells in the touch domes of guard hairs. To investigate the consequences of Sox2 ablation in skin we deleted Sox2 in DP cells via Blimp1Cre and in Merkel cells via K14Cre. Loss of Sox2 from the DP did not inhibit hair follicle morphogenesis or establishment of the dermis and hypodermis. However, Sox2 expression in the DP was necessary for postnatal maintenance of awl/auchene hair follicles. Deletion of Sox2 via K14Cre resulted in a decreased number of Merkel cells but had no effect on other epithelial compartments or on the dermis. The reduced number of Merkel cells did not affect the number or patterning of guard hairs, nerve density or the interaction of nerve cells with the touch domes. We conclude that Sox2 is a marker of two distinct lineages in the skin and regulates the number of differentiated cells in the case of the Merkel cell lineage and hair follicle type in the case of the DP.

Introduction

The transcription factor Sox2 is involved in maintenance of the early, pluripotent stem cells of the eipiblast (Avilion et al., 2003) and in re-establishing pluripotency in postnatal cell types (Takahashi and Yamanaka, 2006). Sox2 is essential for central nervous system (CNS) development and maintenance of neural stem cells (Pevny and Nicolis, 2010). Sox2 is also expressed in adult stem cells and progenitors and plays a crucial role in tissue regeneration in various organs (Arnold et al., 2011).

Sox2 is expressed in the dermal papilla cells of guard/awl/auchene hair follicles (Driskell et al., 2009) and in the dermal sheath cells of some hair follicles (Laga et al., 2010). Dermal papillae are specialised clusters of fibroblasts at the base of each hair follicle that regulate follicle development and cycling via reciprocal signalling with the overlying epidermal cells (Millar, 2002; Driskell et al., 2011). Depletion of Sox2-positive DP cells prevents formation of awl/auchene hair follicles in skin reconstitution assays (Driskell et al., 2009). When Sox2-positive dermal cells are cultured and subsequently grafted into mice they retain their identity, suggesting that they represent a distinct dermal lineage (Driskell et al., 2012b). In those assays Sox2-positive cells not only contribute to the DP but can also be more widely distributed in the dermis (Driskell et al., 2012b), consistent with previous reports that Sox2-positive dermal cells are multipotent Skin Derived Precursors (SKPs) (Toma et al., 2001; Fernandes et al., 2004; Biernaskie et al., 2009).

Within the epidermis Sox2 is expressed in a small population of mechanosensory cells known as Merkel cells (Haeberle et al., 2004; Driskell et al., 2009). These neuroendocrine cells are clustered in the epidermal basal layer adjacent to guard hairs, and constitute touch domes (Lumpkin and Caterina, 2007; Lumpkin et al., 2010). Merkel cells are excitable, express voltage-gated ion channels and are capable of calcium-induced calcium release (Piskorowski et al., 2008; Haeberle, 2004). They also express simple keratins (K8, 18 and 20), neuropeptides and presynaptic machinery proteins (such as Rab3c), as well as transcription factors involved in neuronal cell fate determination (Haeberle et al., 2008). Merkel cells are postmitotic, terminally differentiated cells that are derived from keratin 14-positive cells in the epidermal basal layer that downregulate keratin 14 on differentiation (Van Keymeulen et al., 2009; Woo et al., 2010; Morrison et al., 2009).

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In view of the key contributions of DP cells and Merkel cells to skin function and the observation that Sox2 is a marker of SKPs, we have investigated the consequences of deleting Sox2 in the DP and Merkel cell compartments.

Material and methods

Transgenic mice

All experiments were approved by King's College London, Cambridge University and Cancer Research UK local ethics committees and performed under the terms of a UK government Home Office licence. Sox2fl/fl mice, in which flox sequences flank the Sox2 locus (Favaro et al., 2009), were kindly provided by Silvia Nicolis. CAGCATeGFP, Blimp1Cre and Blimp1GFP mice have been described previously (Kawamoto et al., 2000; Ohinata et al., 2005). NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) immunodeficient mice were acquired from the Jackson Laboratory. K14Cre mice were a kind gift of Michaela Frye (Driskell et al., 2012a) and were originally obtained from the Jackson Laboratory.

Flow cytometry

Flow cytometry was performed on dermal preparations as described previously (Jensen et al., 2010) using a Cyan Flow Analyser. CD133-APC (eBiosciences) and eCadherin-647 antibodies (eBiosciences) were used at the manufacturer's recommended concentrations. Analysis of flow cytometry data was performed using FlowJo software.

Gating criteria were as follows. Debris was gated out using forward and side scatter plots. Doublets and dead cells were also gated out and analysis was performed on live cells using GFP and APC channels. Gating for positively labelled cells was performed against negative control samples to less than 0.5% background.

Histology, whole mounts and immunostaining

Preparation and immunostaining of conventional cryosections (5–30 μm thick) and whole mounts of tail epidermis, back skin and whisker pad were performed as described previously (Driskell et al., 2009). Back skin horizontal whole mounts (100 μm thick) were prepared and immunostained as described by Driskell et al. (2012b).

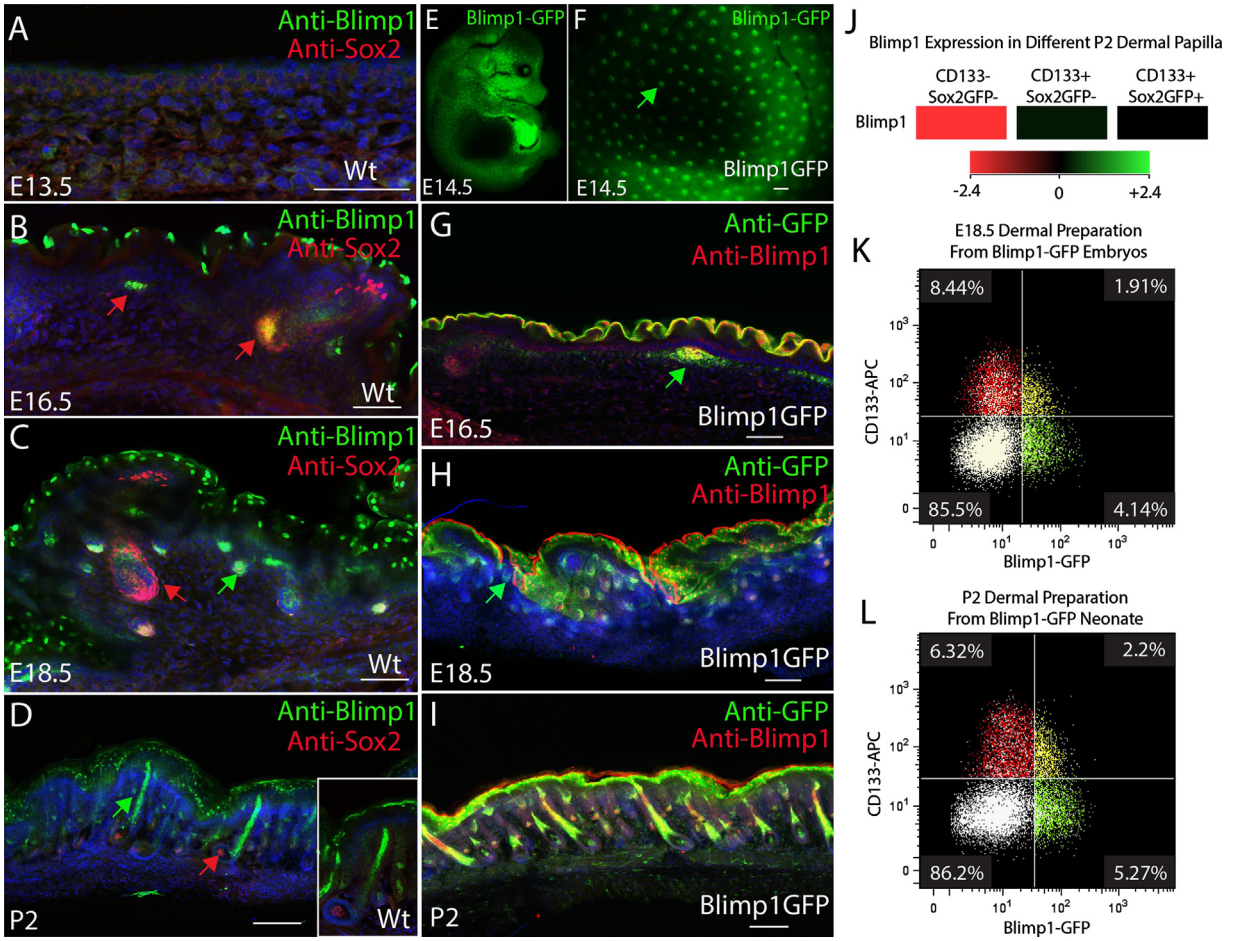

Fig. 1. Blimp1 is expressed by dermal papilla cells during skin morphogenesis. (A–D) Cryosections of E13.5-P2 skin labelled with antibodies to Sox2 and Blimp1. Note Blimp1 expression in DP of guard/awl/auchene hair follicles (red arrows) and zigzag hair follicles (green arrows) and in the outermost epidermal cell layers (B–D). (E–I) GFP expression under the control of Blimp1 regulatory elements colocalizes with endogenous Blimp1 during morphogenesis of guard/awl/auchene (E–G) and zigzag (H–I) hairs. Arrows show dermal condensates (F, G) and dermal papilla (H). (J) Blimp1 mRNA was detected in guard/awl/auchene (CD133+Sox2GFP+) and zigzag (CD133+Sox2GFP−) dermal papilla cells, but not in dermal fibroblasts (CD133−Sox2GFP−). (K, L) Flow cytometry of single cell suspensions of E18.5 (H) and P2 (I) dermal cells, showing co-expression of the dermal papilla marker CD133 with Blimp1GFP. Cells in each quadrant are labelled with a different colour for ease of visualisation. Data are representative of at least $N=3$ biological replicates. Scale bars: (A, B) 1 mm, (C–G) 100 μm.