State-dependent signaling by $Ca_v1.2$ regulates hair follicle stem cell function

Gozde Yucel,[1] Banu Altindag,[1] Natalia Gomez-Ospina,[1] Anshul Rana,[2] Georgia Panagiotakos,[2] Maria Fernanda Lara,[1] Ricardo Dolmetsch,[2] and Anthony E. Oro[1,3]

[1]Program in Epithelial Biology, [2]Department of Neurobiology, Stanford University, School of Medicine, Stanford, California 94305, USA

The signals regulating stem cell activation during tissue regeneration remain poorly understood. We investigated the baldness associated with mutations in the voltage-gated calcium channel (VGCC) $Ca_v1.2$ underlying Timothy syndrome (TS). While hair follicle stem cells express $Ca_v1.2$, they lack detectable voltage-dependent calcium currents. $Ca_v1.2^{TS}$ acts in a dominant-negative manner to markedly delay anagen, while L-type channel blockers act through $Ca_v1.2$ to induce anagen and overcome the TS phenotype. $Ca_v1.2$ regulates production of the bulge-derived BMP inhibitor follistatin-like1 (Fstl1), derepressing stem cell quiescence. Our findings show how channels act in nonexcitable tissues to regulate stem cells and may lead to novel therapeutics for tissue regeneration.

Supplemental material is available for this article.

Received February 23, 2013; revised version accepted May 9, 2013.

Multipotent adult stem cells like those in the hair follicle possess the capacity for programmed organ replacement and carry the promise of induced organ repair in response to injury or damage (Li and Clevers 2010; Bermingham-McDonogh and Reh 2011). In many tissues, multipotent stem cells are found within specific tissue niches of support cells. These niches contain specific extrinsic and intrinsic cues and act to provide regulatory signals that help proliferation or differentiation (Fuchs and Segre 2000). While the relationship between stem cells and niches are known, the controls that regulate the interactions between the cells are an area of active investigation. The murine hair follicle is a great system to study adult stem cells because of its cycle dependency and relatively short switches between growth and destruction phases. Hair follicles have two parts: a permanent part that consists of sebaceous glands and the stem cell-containing or bulge region and the lower or dynamic part that goes through genetically controlled cycles of active growth (anagen), destruction (catagen), and resting phases (telogen) (Hardy 1992).

[*Keywords*: hair follicle stem cells; bulge; calcium channel; VGCC]
[3]Corresponding author
E-mail oro@stanford.edu
Article is online at http://www.genesdev.org/cgi/doi/10.1101/gad.216556.113.

While excitable cells respond to electric signals mediated by voltage-gated calcium channels (VGCCs) (Halling et al. 2006; Striessnig et al. 2010; Bidaud and Lory 2011), the role of VGCCs in nonexcitable cells such as adult stem cells remains poorly understood. VGCCs employ three well-characterized states in regulating calcium in response to strong membrane depolarization (Catterall 2000): a nonconducting closed state, a conducting open state that allows calcium entry, and a closed inactivated state that follows the open state (Supplemental Fig. S1A). VGCCs enter the inactivated state following prolonged depolarization or elevations of intracellular calcium or by binding to channel antagonists such as the dihydropyridine or phenylalkylamine class of L-type channel inhibitors (Catterall 2000). The glycine-to-arginine mutation at position 406 that causes Timothy syndrome (TS) dramatically reduces L-type channel inactivation (Splawski et al. 2004) and therefore provides an opportunity to investigate the role of channel inactivation in various cellular processes.

Here, we investigate the baldness associated with the dominant L-type VGCC $Ca_v1.2$ mutation underlying TS (Online Mendelian Inheritance in Man [OMIM] 601005) (Splawski et al. 2004; Bidaud and Lory 2011). We found that hair follicle bulge stem cells, but not differentiating cells, express $Ca_v1.2$ but lack significant voltage-dependent calcium currents, as determined by bulge cell patch clamping and calcium imaging analyses. Expression of $Ca_v1.2^{TS}$ and loss of the channel in hair follicle bulge stem cells results in markedly delayed anagen, indicating that the TS phenotype acts in a dominant-negative manner. In contrast, treatment with L-type channel blockers that induce channel inactivation cause an early entry to anagen and can reverse the TS phenotype. In additional experiments, we found that $Ca_v1.2$ regulates the production of the bulge-derived BMP inhibitor follistatin-like1 (Fstl1), which acts to inhibit stem cell quiescence. We conclude that $Ca_v1.2$ provides a calcium-independent signal in hair follicle stem cells that inhibits quiescence and promotes tissue regeneration.

Results and Discussion

Because VGCCs are widely expressed in nonexcitable tissues such as hair and TS individuals display a marked delay in hair growth for the first 2 yr of life (Splawski et al. 2004), we investigated how VGCCs such as $Ca_v1.2$ regulate hair growth. In the murine hair, $Ca_v1.2$ protein is not ubiquitously expressed throughout the tissue, but rather is highly expressed in the bulge stem cells as compared with interfollicular epithelium (Greco et al. 2009) and colocalizes with the outer bulge marker CD34 (Fig. 1A). As diversified channel properties arise from $Ca_v1.2$ alternate splicing (Gray et al. 2007), we compared the bulge $Ca_v1.2$ isoform with that found in excitable tissues such as neurons. RT–PCR of *CACNA1C* ($Ca_v1.2$) exons in FACS-sorted $CD34^+$ $\alpha6^+$ bulge stem cells (B) (Nowak and Fuchs 2009) demonstrated a full-length channel isoform that also exists in P1 (postnatal day 1) mouse brains (Fig. 1B).

To determine whether these full-length isoforms carry calcium into cells, we performed electrophysiology to study FACS-isolated bulge stem cells from K15-EGFP mice (Morris et al. 2004). We used whole-cell patch clamping to