CELL REGENERATION

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# Genetic reporter analysis reveals an expandable reservoir of OCT4+ cells in adult skin

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## Abstract

The transcription factor *Oct4* (*Pou5f1*) is a critical regulator of pluripotency in embryonic and induced pluripotent stem cells. Therefore, *Oct4* expression might identify somatic stem cell populations with inherent multipotent potential or a propensity for facilitated reprogramming. However, analysis of *Oct4* expression is confounded by *Oct4* pseudogenes or non-pluripotency-related isoforms. Systematic analysis of a transgenic *Oct4-EGFP* reporter mouse identified testis and skin as two principle sources of $Oct4^+$ cells in postnatal mice. While the prevalence of $GFP^+$ cells in testis rapidly declined with age, the skin-resident $GFP^+$ population expanded in a cyclical fashion. These cells were identified as epidermal stem cells dwelling in the stem cell niche of the hair follicle, which endogenously expressed all principle reprogramming factors at low levels. Interestingly, skin wounding or non-traumatic hair removal robustly expanded the $GFP^+$ epidermal cell pool not only locally, but also in uninjured skin areas, demonstrating the existence of a systemic response. Thus, the epithelial stem cell niche of the hair follicle harbors an expandable pool of Oct4+ stem cells, which might be useful for therapeutic cell transfer or facilitated reprogramming.

**Keywords:** Oct4, Epidermal stem cells, Skin, Transgenic reporter mice

## Background

Regenerative cell therapy for terminally differentiated organs damaged by disease requires multipotent stem cell reservoirs, preferably from autologous sources. The POU-domain transcription factor *Oct4* (*Pou5f1*) is an important regulator of pluripotency in embryonic stem (ES) and induced pluripotent stem (iPS) cells [1]. The latter arise during re-programming from clones re-expressing endogenous *Oct4* [2], while under specific culture conditions ectopic expression of *Oct4* alone in primary somatic cells is sufficient for reprogramming into iPS or multilineage progenitors [3,4]. Therefore, endogenous *Oct4*+ stem cells might either display inherent multipotent differentiation potential or serve as a source for minimal modification approaches to generate iPS with high efficiency. However, analysis of *Oct4* expression is confounded by false-positive results due to *Oct4* pseudogenes or non-pluripotency-related, non-nuclear OCT4B isoforms, explaining contradictory reports on *Oct4* expression in several somatic stem cell populations [5].

The hair follicle, as part of the protective and self-renewing mammalian epidermis, is one of the few organs that undergoes constant cycles of degeneration and regeneration throughout life. It contains an epidermal stem cell niche harboring multipotent epidermal stem cells, which can be mobilized to regenerate the new follicle with each hair cycle or in damaged skin during wound repair [6]. Multipotent epidermal stem cells not only give rise to new epidermis and hair when grafted but are able to correct inherited skin disease in humans and differentiate into all principle tissue lineages in culture [6-9], while a population of skin stem cells can contribute to skeletal muscle fiber regeneration in muscle dystrophy after cell transplantation [10]. Furthermore, a poorly characterized subset of cells isolated from human hair follicles has been shown to express *Oct4* and to display multipotent behavior in vitro [11]. Our objective was to identify adult somatic stem cells expressing *Oct4* using a well characterized

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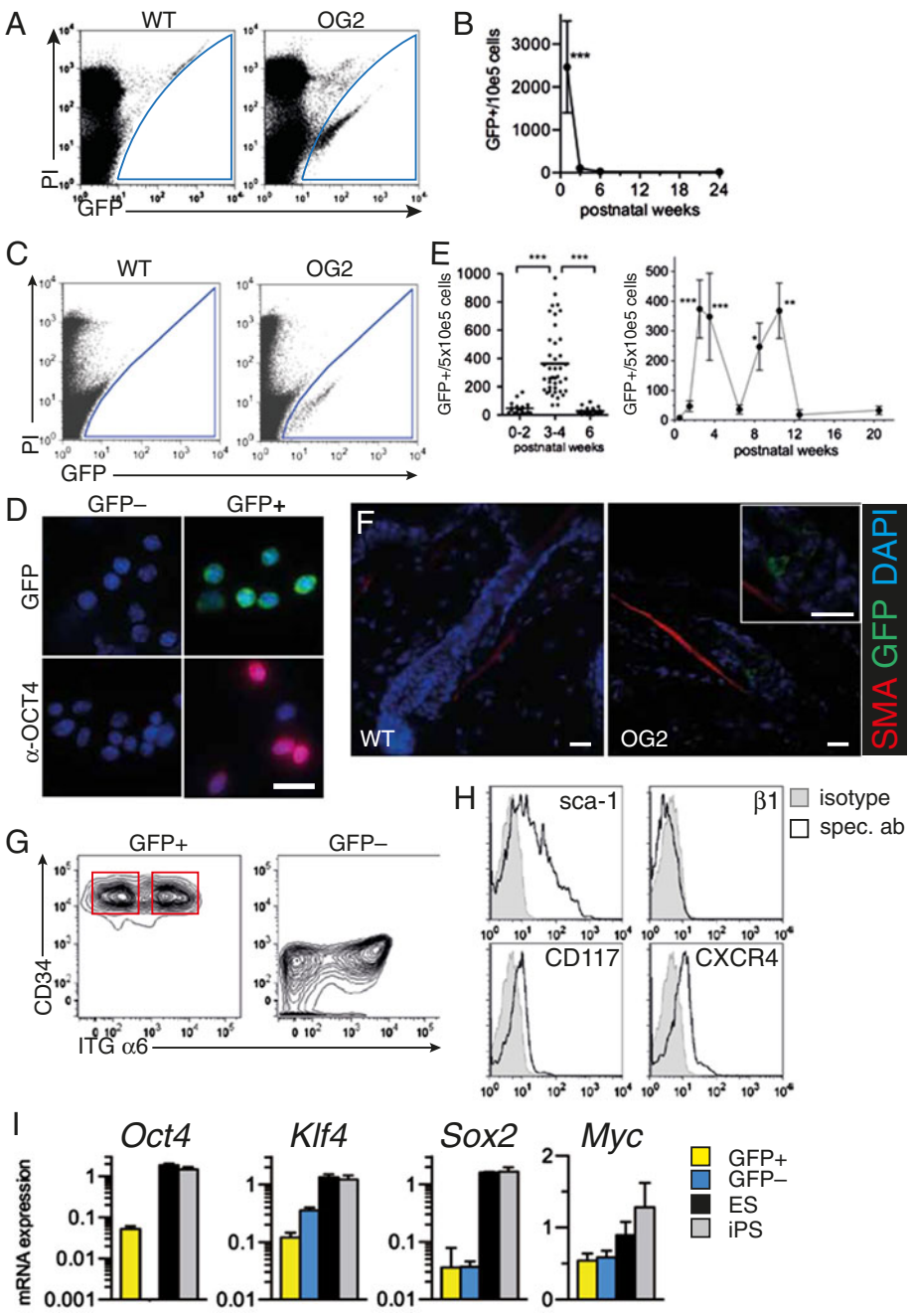


**Figure 1 Characterization of GFP$^+$ cell populations in testis and skin in Oct4-Gfp (OG2) reporter mice. (A)** Flow cytometry of cell suspensions from 1 wk old neonatal testis with compensation for autofluorescence by plotting FL1 against FL2. Dead cells are stained with propidium iodide (PI). **(B)** Timecourse analysis by flow cytometry of GFP + cells in testis at 0–2 wks, 3–4 wks, 6 wks and 24 wks of age. P vs 0–2 wks. **(C)** Representative FACS analysis and GFP$^+$ gating of cell suspensions from skin. **(D)** Fluorescence microscopy of sorted GFP$^+$ and GFP$^-$ cells for GFP, anti-OCT4 antibody staining (red) and nuclear dye (DAPI, blue). **(E)** GFP$^+$ skin cells by FACS in different age groups (left), n = 36-45. Cyclic expansion of the GFP$^+$ cell pool over time (right). n = 9-30. P vs 0.5 wks. **(F)** Immunostaining of hair follicle bulge region against smooth muscle α–actin (SMA) and GFP. Insert from same image in higher magnification. **(G)** Flow cytometry of GFP+ or GFP- gated cells from skin. CD34$^+$ ITGα6$^{hi}$ and CD34$^+$ ITGα6$^{lo}$ populations are indicated. **(H)** Representative flow cytometry profile of GFP$^+$ gated cells stained with specific antibodies (spec. ab) or isotype control (isotype). **(I)** mRNA expression ($2^{\Delta Ct}$) by quantitative real-time PCR of pluripotency factors from sorted skin cells or cultures of iPS and ES cells. n = 3-4. Scale bar: 20 um. Magnification: **(D)** 640, **(F)** 200, 640 (insert).