Long Live Sox2: Sox2 Lasts a Lifetime

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Sox2 is a key transcription factor required for the maintenance of pluripotency in embryonic cells and morphogenesis of several epithelia. In this issue of *Cell Stem Cell*, Arnold et al. (2011) demonstrate that Sox2 marks long-lived adult stem cells to ensure homeostasis in a broad range of adult tissues.

Sox2 is a member of the SRY-related High Mobility Group transcription factor family expressed in pluripotent embryonic stem cells (ESCs). Sox2 is part of the core of the pluripotency network that acts together with other transcription factors, such as Oct4 or Nanog, to promote pluripotency (Jaenisch and Young, 2008). The essential role of Sox2 during early embryonic development has been demonstrated by the failure of Sox2 null embryos to develop further than the epiblast stage (Avilion et al., 2003). The role of Sox2 in controlling the pluripotent state is also demonstrated by the higher efficiency of induced reprogramming to pluripotency of somatic cells when Sox2 is coexpressed with Oct4, Klf4, and c-Myc in a cocktail of reprogramming factors (Jaenisch and Young, 2008).

Sox2 is expressed at later stages of embryonic development in different tissues and organs (Figure 1B). Using Sox2-GFP knockin mice, Ellis and colleagues investigated the pattern of Sox2 expression in the developing embryo and adult mice. They found that Sox2 is expressed in the neural tube and within the proliferating neuronal progenitors during embryonic development and in adult mice (Ellis et al., 2004). Changing the dose of Sox2 during development resulted in major defects of neurogenesis, highlighting the role of Sox2 in preventing neuronal progenitor differentiation (Graham et al., 2003). Sox2 is expressed in the foregut endoderm, in particular in the prospective esophagus and anterior stomach. Hypomorphic Sox2 mutations in mice recapitulate the pathological conditions associated with the disorder caused by Sox2 mutations in humans, called anophthalmia-esophageal-genital syndrome, with symptoms including mircrophthalmia (small eyes) and tracheo-esophageal fistula (communication between the trachea and the esophagus) (Que et al., 2007). In the absence of Sox2, cells of the anterior stomach ectopically express genes of the glandular stomach and intestine, suggesting that Sox2 may establish the boundary between the anterior and the glandular stomach (Que et al., 2007). Absence of Sox2 also results in the abnormal differentiation of tracheal cells and lung epithelial cells, and postnatal expression of Sox2 is required to sustain the homeostasis of the trachea and the ability of tracheal cells to regenerate the epithelium upon injury (Que et al., 2009). All together, these data indicate that Sox2 is expressed at different stages of embryonic development and persists in certain adult tissues. However, no systematic analysis of the full pattern of Sox2 expression in adult tissues has been performed and, more importantly, the identity and function of Sox2-expressing cells in adult tissues remain unclear.

To elucidate the functional role of Sox2 in adult tissue, Arnold and colleagues first performed a systematic survey of Sox2 expression in adult tissues using Sox2-GFP knockin mice and Sox2 immunostaining. They confirmed the previously reported expression of Sox2 in neurogenic zones of the brain, retina, tongue, trachea, and bronchiolar epithelium. In addition, they also identified, for the first time, Sox2 expression in testis, cervix, lens epithelium, glandular stomach, and squamous epithelia of the esophagus (Figure 1).

The broad range of Sox2 expression in these renewing tissues prompted the authors to determine more precisely the contribution of Sox2-expressing cells to tissue homeostasis. To this end, they generated novel genetically engineered mice, harboring a tamoxifen (TAM)-inducible Cre allele (CreER) or a suicide gene (Herpes Simplex Thymidine Kinase, or HSTK) in the Sox2 locus, in order to perform temporally regulated lineage tracing and fate mapping analysis of Sox2-expressing cells, as well as lineage ablation of $Sox2^+$ cells following drug administration. TAM administration to adult Sox2-CreER/ROSA26-lsl-EYFP mice resulted in the initial labeling of cells within the testis, pylorus and corpus of the glandular stomach, and the basal layer of the lens, as well as in other tissues in which Sox2 was previously shown to be expressed, including the tongue, esophagus, forestomach, cervix, and anus (Figure 1C). Several months after TAM administration, all these tissues were still strongly YFP^+. The long-term persistence and expansion of Sox2-derived cells in these lineage-tracing experiments are consistent with the targeting of long-lived stem cells within these tissues. $Sox2^+$ adult stem cells can be either unipotent (e.g., germ and lens stem cells) or multipotent at the population level, such as those in the stomach, where $Sox2^+$ stem cells differentiate into parietal, neuroendocrine, and glandular mucin-producing cells of the glandular stomach. Lineage tracing experiments recently showed that Lgr5 is also expressed by stem cells of the adult stomach (Barker et al., 2010). However, Sox2 and Lgr5 seem to mark two separate populations of cells. Further studies will be needed to determine whether $Sox2^+$ and $Lgr5^+$ stem cells are hierarchically connected or represent purely independent classes of stomach stem cells. Furthermore, it is unclear whether all $Sox2^+$ cells are equipotent adult stem cells that balance self-renewal and differentiation in a stochastic manner, or alternatively, whether only a subpopulation of $Sox2^+$ cells are bona fide stem

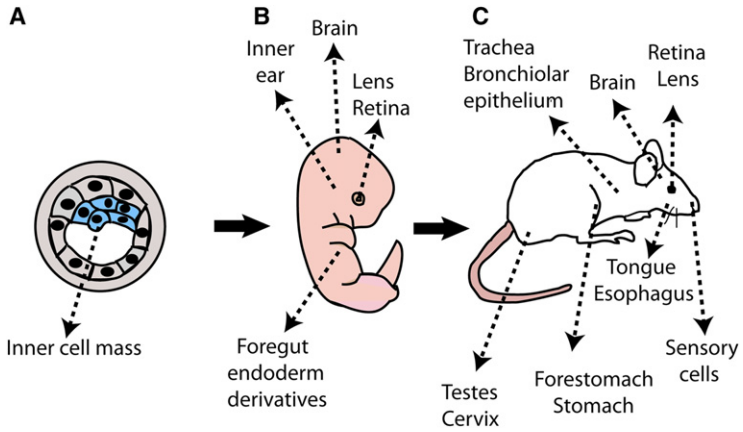

Figure 1. Sox2 Expression in Pluripotent Cells, Embryonic Progenitors, and Adult Stem Cells
Sox2 is expressed in the inner cell mass of the blastocyst (A) and in the developing embryo. At E15.5, Sox2 is expressed in the foregut endoderm derivatives, as well as in neuronal, inner ear, retina, and lens progenitors (B). In adult mice, Sox2 is expressed in tissue stem cells of stratified and glandular epithelia of ectodermal and endodermal origin, including the glandular stomach, the esophagus, the tongue, the brain, the trachea, and the bronchiolar epithelium, as well as in sensory cells (Merkel and taste bud cells) and spermatogonial stem cells (C).

cells. It has been proposed that adult Sox2$^+$ neuronal stem cells derive from embryonic Sox2$^+$ progenitors (Suh et al., 2007). TAM administration to Sox2-CreER/ROSA26-lsl-EYFP pregnant mice between E13.5 and E15.5 induced YFP expression in the prospective adult tissues expressing Sox2 (Arnold et al., 2011), consistent with the notion that adult Sox2$^+$ stem cells arise from embryonic Sox2$^+$ progenitors.

To further underscore the long-term renewal potential of Sox2$^+$ stem cells, the authors isolated spermatogonia based on Sox2-GFP and c-kit expression and transplanted different cell populations (Sox2-GFP$^+$/c-kit$^-$, Sox2-GFP$^+$ c-kit$^+$, and Sox2-GFP$^-$) into the testes of infertile male mice. They showed that only Sox2-GFP$^+$/c-kit$^-$ cells contain spermatogonial stem cells, because only these cells, but not Sox2-GFP$^-$ or Sox2$^+$/c-kit$^+$ cell populations, could restore fertility by repopulating seminiferous tubules and differentiated mature sperm (Arnold et al., 2011).

Finally, to further substantiate the function of Sox2-expressing cells in maintaining homeostasis of different adult tissues, the authors performed lineage ablation of Sox2$^+$ cells by administrating ganciclovir (GCV) to adult Sox2-HSTK knockin mice. GCV is transformed into a toxic metabolite that kills cells expressing HSTK. As expected, GCV administration resulted in massive apoptosis in Sox2-expressing cells, inducing major defects of tissue homeostasis that lead to the death of the mice after 2 weeks of treatment. When the treatment was withdrawn after 1 week, the pathological phenotype reverted to normal, concomitant with the replenishment of Sox2$^+$ progenitors in these tissues (Arnold et al., 2011). This remarkable study demonstrates that Sox2 is expressed in, and regulates the function of many types of, adult stem cells. The conserved role of Sox2 in regulating the function of a broad range of stem cells, including pluripotent stem cells, various tissue-specific embryonic progenitors, and adult stem cells, suggests that Sox2 may act as a common "stemness" gene regulating the self-renewal potential of many different classes of stem cells. It will be important to define whether Sox2 functions in these various stem cells by regulating common or different sets of genes. It will also be important to determine whether the amplification of Sox2 found in esophageal and lung squamous cell carcinoma (Bass et al., 2009) promotes self-renewal and stemness of tumor cells and whether Sox2-expressing cancer cells represent the so-called cancer stem cells of these tumors.

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