Identification and functional characterization of Sox2-target genes involved in the self-renewal and differentiation of neural stem cells cultured from the mouse brain

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The Sox2 gene encodes a transcription factor active in neural stem/progenitor cells (NSCs) in the developing vertebrate central nervous system (CNS). Heterozygous Sox2 mutations in humans cause a characteristic spectrum of CNS abnormalities. To understand the role of Sox2 in neural development, we previously generated Sox2 conditional KO mutations in mouse, that allowed us to observe an important function for Sox2 in the maintenance of NSCs self-renewal, in long-term *in vitro* cultures derived from P0 mouse forebrain, as well as *in vivo* (e.g. in hippocampus). In *in vitro* cultures, Sox2-ablated NSCs self-renew for several passages like the wild-type ones, but then undergo progressive exhaustion. We found that, upon differentiation, they also generate reduced numbers of neurons, with reduced arborization. Sox2 can regulate its targets by controlling long-range interactions between genes and distal enhancers, which regulate gene expression; indeed, many of these interactions are lost in Sox2-mutant cells. By RNAseq, we identified genes that are downregulated following Sox2 ablation. To test their role as mediators of Sox2 functions, we re-introduced some of them into Sox2-deleted cells via lentiviral vectors, to test if they can rescue long-term self-renewal, and neuronal differentiation. The most downregulated gene in mutant, compared to wild-type cells, is Socs3 (Suppressor Of Cytokine Signalling 3). Its overexpression rescues the ability of mutant cells to grow long-term, and may partially rescue the neuronal differentiation defect. Other genes downregulated in Sox2-mutant NSCs include key regulators of cell proliferation, like c-Fos, Jun and Egr2. We found that c-Fos is the most important to rescue the ability of Sox2-mutant cells to grow long-term. Having found that Fos acts downstream to Sox2 in the maintenance of NSCs self-renewal, we conducted further functional analysis to understand if Fos itself is required to maintain NSCs, in the presence of wild-type Sox2. We thus mutated the endogenous Fos gene, using the CRISPR/Cas9 system. We tested the ability of NSCs to self-renew generating stem cells capable of giving rise to clonal progeny. A small number of clones growing from mutagenized cells could be progressively expanded and continued growing, while the majority stopped growing and died out at previous stages, compared to clones growing from cells transduced using a control virus. These data indicate that mutation of the endogenous Fos gene progressively reduces the rate of cell growth. This is in agreement with a functional role for Fos in brain-derived NSCs maintenance.