SOX2 mutagenesis in human Pluripotent Stem Cells through the CRISPR-Cas9 Homology-Directed Repair pathway

[1]Sara E. Barilà, [1]Veronica Krenn and [1]Silvia K. Nicolis

Correspondence: s.barila1@campus.unimib.it

[1]Department of Biotechnology and Biosciences, University of Milano-Bicocca, piazza della Scienza 2, 20126 Milan, Italy

Keywords: Sox2, human Pluripotent Stem Cells, CRISPR-Cas9

Abstract:

Sox2 is a transcription factor essential for embryonic development from the very first stages. It is expressed in the inner cell mass of the mouse embryo and its presence is so crucial that the ablation by a homozygous null mutation results in the arrest of embryonic development, just after the embryo implants in the uterus [1].

Later in development, Sox2 expression marks the forming nervous system, from neural induction onwards. Heterozygous loss of function mutations in the Sox2 gene lead to a rare human disorder characterized by micro- or anophthalmia, hypoplasia of the hippocampus, cognitive impairment, and motor defects. Conditional knock-out (cKO) mice were generated to ablate Sox2 in different areas of the developing nervous system and at different developmental time points; these models revealed an essential role of Sox2 in the development of specific areas of the brain, such as the hippocampus, the Medial Ganglionic Eminence (MGE) and the thalamus [2].

Here, we aim to generate a new experimental model for the study of SOX2 roles in embryonic development and pathogenesis by targeted SOX2 mutagenesis in human Pluripotent Stem Cells (hPSCs). We will use the CRISPR-Cas9 system via homology-directed repair to introduce a Sox2 mutation (rs750091101 C/A) observed in humans with Syndromic microphthalmia-3 (MCOPS3), generating an in-frame stop codon leading to a premature arrest of translation. Afterwards it will also be possible to induce this mutated hPSCs to generate organoids of different brain areas, thereby studying SOX2 function in a complex model mirroring important aspects of human brain development and cell type composition. With this project we hope to deepen the already obtained results and shed light on still unknown aspects of SOX2 biological functions.

1. Avilion, A.A.; Nicolis, S.K. et al. Multipotent cell lineages in early mouse development depend on SOX2 function. Genes Dev. 2003, 17, 126–140.
2. Mercurio, S.; Serra, L. et al. Deconstructing Sox2 Function in Brain Development and Disease. Cells 2022, 11, 1604.