FOXO3-engineered human ESC-derived vascular cells promote vascular protection and regeneration

With the support by the National Natural Science Foundation of China and the Chinese Academy of Sciences, the research team led by Prof. Liu GuangHui (刘光慧) at the CAS National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, generated the world's first genetically enhanced human vascular cells by targeting a single longevity gene, *FOXO3*. This study was recently published in *Cell Stem Cell* (2019) as a cover story.

Regenerative medicine is the process of replacing, engineering or rejuvenating human cells, tissues or organs to restore normal human function. Transplanting cells obtained via *in vitro* differentiation into the lesion sites promotes the regeneration of damaged tissues and restores the homeostasis and function of tissues and organs. However, the outcome of existing therapeutic approaches is far from optimal. Safety concerns regarding stem cells, such as the risk of gene-editing-associated tumorigenesis are a major barrier to the widespread application of this technology.

FOXO3, an evolutionarily conserved longevity factor, functions as an important regulator to delay cellular senescence, resist various stresses and maintain cardiovascular homeostasis. Activation of FOXO3 counteracts malignant transformation of cells by promoting the expression of tumor suppressor genes and maintaining genome stability.

Liu's group generated the genetically enhanced human vascular cells via gene-editing technology and revealed a new mechanism for the long-lived protein FOXO3 in maintaining human vascular homeostasis through six years of effort. In this study, using genome editing, they replaced two nucleotides in exon 3 of the *FOXO3* gene in human cells that inhibited the phosphorylation and subsequent nuclear export and degradation of FOXO3, thus promoting the nuclear translocation of the FOXO3 protein and expression of its target genes.

FOXO3-enhanced vascular cells exhibited improved self-renewal and increased resistance to oxidative injury compared with those of wildtype cells. When tested in a therapeutic context, they promoted vascular regeneration in a mouse model of ischemic injury and were resistant to tumorigenic transformation both *in vitro* and *in vivo*. Mechanistically, constitutively active FOXO3 conferred cytoprotection by transcriptionally downregulating *CSRP1*.

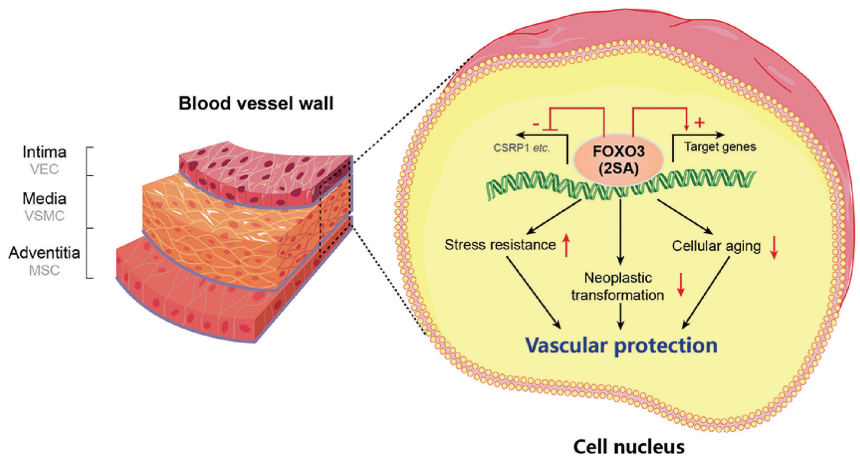

Figure A putative model describing the role of FOXO3 enhancement in the maintenance of vascular cells homeostasis.

The findings prove the feasibility of using gene-editing strategies to obtain high-quality, safe human vascular cell grafts and make it possible to scale up and standardize the generation of such cells for therapeutic use. This study provides a promising option for future regenerative medicine.