CRISPRa-mediated targeting of FOXP3 gene regulatory regions

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Forkhead box P3+ (FOXP3+) regulatory T cells (Tregs) are a subset of lymphocytes, critical for the maintenance of immune homeostasis. Loss-of-function mutations of the FOXP3 gene in animal models and humans results in loss of differentiation potential into Treg cells and are responsible for several immune-mediated inflammatory diseases. Strategies of increasing FOXP3 expression represent a potential approach to increase the pool of Tregs within the lymphocyte population and may be employed in therapies of diverse autoimmune conditions. In the present study, a dCas9 CRISPR-based method was systematically employed to achieve upregulation and sustained high expression of endogenous FOXP3 in mammalian cell lines through targeting of the core promotor and several regulatory regions. Using an activator-domain fusion based dCas9 transcription activator, robust upregulation of FOXP3 was achieved, and an optimal combination of single guide RNAs was selected, which exerted an additive effect on FOXP3 gene upregulation. Simultaneous targeting of FOXP3 and EOS, a transcription factor known to act in concert with FOXP3 in initiating a Treg phenotype, resulted in upregulation of FOXP3 downstream genes CD25 and TNFR2. dCas9-based systems provide great promise in DNA footprint-free phenotype perturbations (perturbation without the risk of DNA damage) to drive development of transcription modulation-based therapies.