Relazione di fine secondo anno di Dottorato in Medicina Molecolare Ciclo XXXII, aa 2017/2018

Università degli Studi di Siena- Dipartimento di Medicina Molecolare e dello Sviluppo
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La sottoscritta Dott.ssa **Enrica Pellegrino**, come previsto dal DD n. 1601/2016, presenta la seguente relazione sulle attività intraprese durante il secondo anno di dottorato.

INTRODUCTION

Conditional gene inactivation in diploid cells can be achieved using the FLIP conditional gene knockout (cKO) approach, a one-step strategy recently described[1] and validated in mouse embryonic stem cells (mESC). The goal of my thesis project is to extend this strategy to human induced pluripotent stem cells (hiPSC). At the same time, to enable drug-inducible conditional mutagenesis, I incorporated two site-specific recombinase systems in the AVVS1 safe harbour locus of human stem cells, CreERT2 and the tetracycline-controlled transcriptional activation of Flp recombinase (rtTA_TRE_FLPO), where Cre or Flp are activated upon treatment of cells with 4 – hydroxytamoxifen (4-OHT) or Doxycycline (Dox), respectively.

METHODS

KOLF2_C1 cells were nucleofected with Cas9 RNP and a donor plasmid to introduce the FLIP cassette into exon 2 of SMAD4. Correct targeting of SMAD4 was determined by PCR genotyping across the 5' and 3' homology arms and sequencing of the non-targeted allele. Biallelic targeting of the FLIP cassette was identified by the absence of a wild-type allele. RNA and protein were collected in mutant and control cells treated with or without 4-OHT or Dox: protein expression was determined by Western Blot and RNA splicing was analysed by Reverse Transcriptase-PCR (RT-PCR). The FLIP-CreERT2/rtTA_TRE_FLPO platform for drug-inducible loss-of-function and reversion studies was generated by engineering the AAVS1 locus[2]

RESULTS

In hiPSC we find that the one-step FLIP strategy is problematic: in the 'non-mutagenic' orientation, I observed hypomorphic expression of the target gene (SMAD4, METTL3, T gene) due to mis-splicing as shown by Western Blot and RT_PCR analysis. Removal of the FLIP cassette with Flp does not restore normal splicing, suggesting that the split exon design is not a viable strategy for human stem cells. I am now testing if targeting the FLIP cassette to an intron of the target gene will preserve wild-type protein expression.

The CreERT2 and rtTA_TRE_FLPO platform for drug-inducible recombination of loxP and FRT sites, respectively, can be applied to many editing applications in human iPSC. My data shows that both inducible Cre and Flp are tightly regulated and efficient, providing a platform for drug-inducible ablation and reversion of gene function in human iPSCs.

REFERENCE

1. Andersson-Rolf A. et al. One-step generation of conditional and reversible gene knockouts. Nat. Methods. 2017
2. Oceguera-Yanez F. et al. Engineering the AAVS1 locus for consistent and scalable transgene expression in human iPSCs and their differentiated derivatives. Methods. 2015

PUBLICATIONS:

- Pizzino G, Irrera N, Galfo F, Oteri G, Atteritano M, Pallio G, Mannino F, D'Amore A, **Pellegrino E**, Aliquò F, Anastasi GP, Cutroneo G, Squadrito F, Altavilla D, Bitto A. Adenosine Receptor Stimulation Improves Glucocorticoid-Induced Osteoporosis in a Rat Model. Front Pharmacol. 2017 Sep 5;8:558.
- Pizzino G, Irrera N, Galfo F, Pallio G, Mannino F, D'amore A, **Pellegrino E**, Ieni A, Russo GT, Calapai M, Altavilla D, Squadrito F, Bitto A. Effects of the antagomiRs 15b and 200b on the altered healing pattern of diabetic mice. Br J Pharmacol. 2018 Feb;175(4):644-655. doi: 10.1111/bph.14113. Epub 2018 Jan 18. PubMed PMID: 29178246; PubMed Central PMCID: PMC5786458.

ABSTRACTS:

- **Enrica Pellegrino**, Jeffrey Thorne, Stefano Landi, William C Skarnes. "Engineering a drug-inducible allelic series in human stem cells". Cold Spring Harbor.
- Justin McDonough, **Enrica Pellegrino**, William C Skarnes. "Improving homology-directed repair efficiency in human stem cells". Cold Spring Harbor.

EDUCATIONAL ACTIVITIES:

- Cold Spring Harbor Genome Engineering: The CRISPR/Cas 9 Revolution meeting. 22-25 August 2018.

Seminar at the Jackson Laboratory

- "Illuminating the chemistry of the skin and the associated microbes". Amina Bouslimani, PhD, University of California, San Diego.
- "Genetic Diagnosis in the Era of the Genomic Medicine". Yoshiko Mito, PhD, Mount Sinai Genomics
- "Advancing our understanding of Huntington's Disease mechanisms through systems biology methods and large-scale genomic data". Jim Rosinski, Ph.D., Scientific Director of Computational Modeling, CHDI Foundation, New York.
- "Exosomes and other Extracellular vesicles in communication between tumors and the immune system". Clotilde Théry, Ph.D., INSERM Director of Research, Institute Curie, Paris, France.
- "Inducible and deterministic programming of human pluripotent stem cells into somatic cell types – The stem cell promise fulfilled?". Mark Kotter, M.D., Ph.D., Cambridge University, UK
- "Multifacted Roles of piRNAs in Gene Regulation". Haifan Lin, Ph.D., Yale University School of Medicine

TRAINING ACTIVITIES ABROAD:

The Jackson Laboratory for Genomic Medicine, Cellular Engineering, Farmington, CT.

Dottoranda
Enrica Pellegrino

Tutor
Prof. Stefano Landi

Co-Tutor
Prof. William Skarnes