Future perspectives using Lysosomal Storage Disease iPSCs models and gene editing therapy

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Introduction

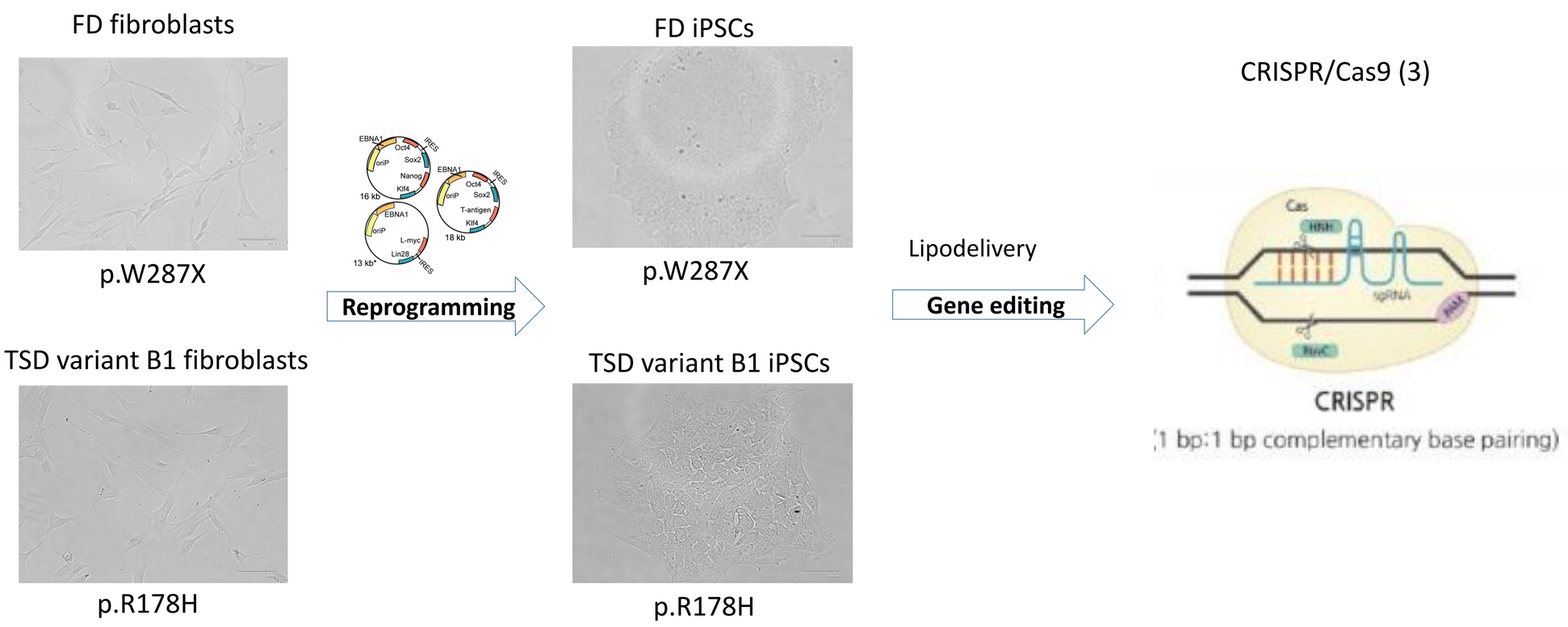
- Lysosomal storage diseases (LSDs) are characterized by accumulation of macromolecules in the late endocytic system. Their collective frequency of 1/5000 live births and are caused by inherited defects in genes that mainly encode lysosomal proteins (1).
- In the Portuguese population, lysosomal storage disorders (LSDs) have a prevalence of 1/4000 live births. Tay Sachs disease (TSD, MIM#272800) variant B1 is one of the most prevalent in the Portuguese population (2). The TSD variant B1 is caused by mutations on the *HEXA* gene (MIM#606869.0006), leading to hexosaminidase A malfunction. The mutation subject of this study, p.R178H (rs28941770), is frequent in specific populations. In the Portuguese it has a carrier frequency of 1:340, and in the North of Portugal it was estimated to be 1:119.
- Fabry disease ((FD, MIM#301500)) is one of the most frequent LSDs, it is caused by mutations on the *GLA* gene (MIM#300644), such as the mutation p.W287X (rs104894839), leading to alpha-galactosidase A impairment.
- Gaucher disease (GD) is also a frequent LSD and it is, usually, due to deficient activity of lysosomal acid beta glucosidase (GBA1, MIM#606463). It has several phenotypic forms (GD1, 230800; GD2, 230900; and GD3; 231000) of which the most severe are neurodegenerative and elude common therapies.

Aim: In our group, we are attempting to use gene editing through CRISPR/Cas9 as a therapeutic tool to correct specific mutations involved in the abovementioned diseases. Our aim is first to obtain induced pluripotent stem cells (iPSCs) derived from these cell lines and then to correct the mutational defects.

Methods and Expected Results

We have generated iPSCs (INSAi001-A, INSAi002-A) (5,4) from Gaucher disease, Fabry disease and Tay Sachs disease patients (Fig.1). The latter iPSCs is undergoing characterization. To perform the CRISPR/Cas9 correction we will design our single-guide RNAs (sgRNAs) on appropriate platforms that enable the selection of the the best sgRNAs, with better in-targets and lower off-targets. The efficacy of transfection will be evaluated using adequate software. Cells with the desired correction will be selected and submitted to sequencing, cytotoxicity assays, and Next Generation Sequencing (NGS) to determine the specificity of CRISPR/Cas9 editing.

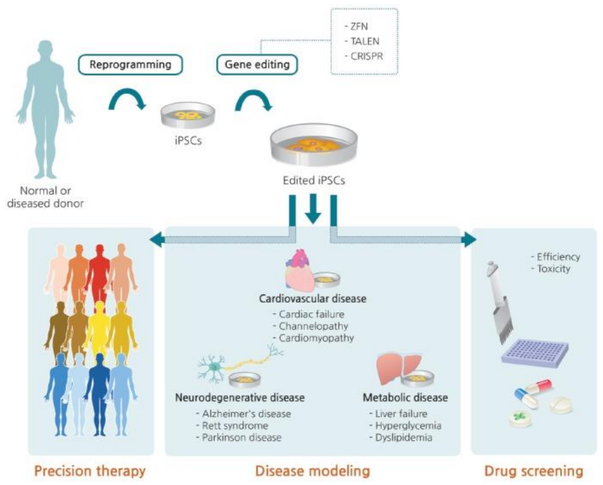
Figure 1: Example of the workflow: from iPSC to gene editing



Conclusions and future perspectives

- ✓ As seen in Fig. 2, the applications of gene editing have great potential in therapeutics
- ✓ Gene editing in monogenic diseases may prove to be an effective therapeutic approach in the near future
- ✓ With this work we hope to achieve a workflow that can be applied to the abovementioned diseases as well as others

Figure 2: From iPSCs to gene editing (3)



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