RaDiChal'21
FINAL

2021 Rare Disease Challenge

Ex vivo cell therapy project of CRISPR HDR based CFTR correction on iPSCs with differentiation to pulmonary ionocytes

CysMode

Affiliation

1 Marmara Üniversitesi Mühendislik Fakültesi Biyomühendislik Bölümü

2 Marmara Üniversitesi Tıp Fakültesi

Gizem Gülfidan[1]
Özge Önlütürk[1]
Esra Yıldırım[1]
Muhammed Fatih Kırcalı[1,2]
Dilan Nisa Yılmaz[1]

Abstract

Cystic fibrosis is a disease that occurs with loss of protein amount or function due to mutations in the CFTR gene (the most common F508del), deterioration of mucus balance and obstruction in the lung airways. Mucolytic agents, washing solutions, anti-inflammatories and antibiotics are used in the management of the disease. In recent years, mutation-specific potentiator, corrector and cell traffic regulators have also been added to the treatment (Ratjen et al., 2015).

Despite all the advances, current treatments are insufficient and life expectancy is limited to 52 years. CRISPR-based gene editing systems are promising to respond to the need for treatment. Among the treatment strategies, ex vivo methods come to the forefront with gene editing easier and more efficient than in vivo methods, enabling post-editing control studies. In addition, ex vivo methods allow the use of induced pluripotent stem cells (iPSC), allowing the use of non-immunogenic readily available cell sources. Among the gene editing tools, the CRISPR HDR system stands out due to its high efficiency (30 times compared to the prime editors) and its usefulness on the F508del mutation (the base editor cannot be used in three nucleotide deletions) (Ensinck et al., 2021).

One of the most important obstacles to the success of iPSC methods is the excess amount of cells that need to be transplanted. The newly discovered pulmonary ionocyte cells in the lung airway epithelium show promise in closing the CFTR deficit with a small amount of cells, with their high CFTR expression per cell and their ability to provide 50% of the CFTR proteins in the tissue despite their low population(Plasschaert et al., 2018). For these reasons; *ex vivo* cell therapy project of CRISPR HDR based CFTR correction on iPSCs with differentiation to pulmonary ionocytes has been prepared for cystic fibrosis.

Projects

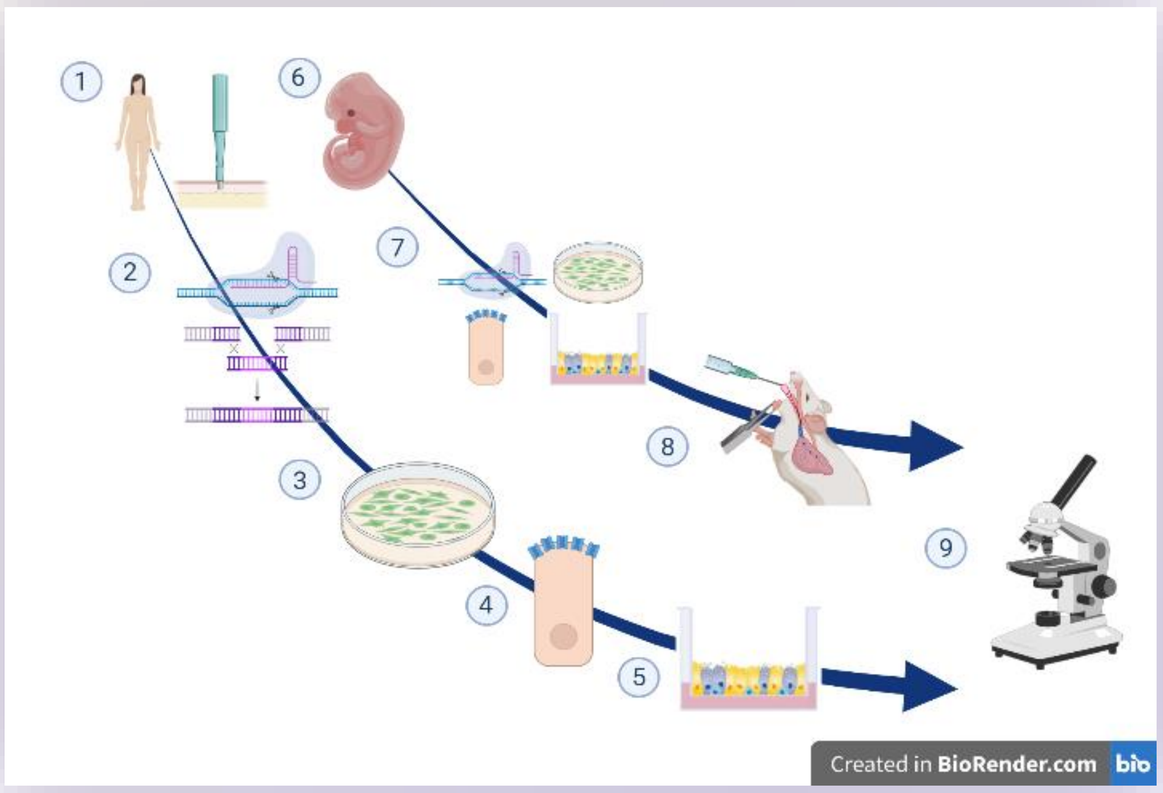

Figure 1: Main steps of the project. (1) Fibroblasts are collected from patient skin via punch biopsy. (2) Fibroblasts are cultured and CFTR is corrected with CRISPR HDR system. (3) Corrected fibroblasts are selected, cultured and induced with Yamanaka Factors to become iPSC, iPSCs are differentiated to lung progenitors. (4) Lung progenitors are differentiated to pulmonary ionocytes with ionocyte specific transcription factors Foxi1 and Ascl3. (5)Transfected cells are cultured on air liquid interface to form pulmonary epithelium rich of ionocytes. (6) Bleomycin resistant non CFTR expressing mouse embryos are sacrificed to obtain embryonic fibroblasts. (7) Steps 2, 3, 4 and 5 are repeated with mouse cells. (8) Non CFTR expressing mouseses' lungs are conditioned with blomycin and pulmonary ionocytes are engrafted using intra-tracheal route. (9) Cell cultures and mouse biopsies are used to assess therapeutic potential of ionocytes with molecular, electrochemical and physiological methods.

Social Impact

@cysmode @CysMode @CysMode

Followers: 150
Likes: 500+
Sharings: 15+
Watchs:850+

Figure 2. Poster of a conference

During the competition period, we tried to give importance to our social responsibility projects as much as the time we allocate to our gene project. In this process, we have organized informative and awareness-raising studies about our target disease, Cystic Fibrosis, and rare diseases. In these studies, we have met with different academicians from Turkey's leading universities, and we have organized seminars and conferences on the history, genetics and little-known aspects of Cystic Fibrosis.

In our first study, which has a special place for us, we organized a podcast in cooperation with Podsonus Media in order to reach people who are not our primary target audience, and in this podcast, we have prepared a broad summary about the disease by talking about the genetics, physiology and treatment methods of Cystic Fibrosis along with the psychology of the patients as a part of our society. In addition to all of them, we came together with the young doctors of the future, aiming to increase the quality of patient-doctor relationship and made a detailed presentation.

All of our work has been aimed at increasing the awareness of rare diseases in general and Cystic Fibrosis in particular, increasing the quality of social life of people with rare diseases and making the society aware of the existence and effects of these diseases in general.

Figure 3. Poster of symposium

Conclusion

The search for a cure for cystic fibrosis continues and new gene editing techniques are gaining importance. In this project, the framework of an *ex vivo* cell therapy protocol that will use newly discovered pulmonary ionocytes derived from iPSCs that are corrected for CFTR using CRISPR Cas9 HDR system is prepared.

Application of this project will advance existing knowledge of differentiation of pulmonary ionocytes and reveal the therapeutic potential for cystic fibrosis.

References

Ratjen, F., Bell, S. C., Rowe, S. M., Goss, C. H., Quittner, A. L., & Bush, A. (2015). Cystic fibrosis. Nature Reviews. Disease Primers, 1(May), 15010. https://doi.org/10.1038/nrdp.2015.10

Ensinck, M., Mottais, A., Detry, C., Leal, T., & Carlon, M. S. (2021). On the Corner of Models and Cure: Gene Editing in Cystic Fibrosis. Frontiers in pharmacology, 12, 662110. https://doi.org/10.3389/fphar.2021.662110

Plasschaert, L. W., Žilionis, R., Choo-Wing, R., Savova, V., Knehr, J., Roma, G., Klein, A. M., & Jaffe, A. B. (2018). A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte. Nature, 560(7718), 377–381. https://doi.org/10.1038/s41586-018-0394-6