Commentary

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Insights in Stem Cells

2018
Vol.4 No.1:2

Correction of *CFTR* gene mutations by genomic edition

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Rec date: December 22, 2017; **Acc date:** February 12, 2018; **Pub date:** February 15, 2018

Citation: Camarasa MV (2018) Correction of *CFTR* gene mutations by genomic edition. Insights Stem Cells Vol.4:No.1:2.

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Cystic fibrosis impairs breathing by dehydrating mucus, causing infection and inflammation cycles which end into premature respiratory failure. Due to the substantial increase in the knowledge of molecular and cellular mechanisms of the disease during past decades, life expectancy has hugely increased up to nearly forty years. Still, the disease severely impacts the quality of life and shortens life expectancy. The ability to generate pluripotent stem cells from accessible tissues as skin, has offered the possibility of developing novel therapies in human models.

Genome editing allows replacing mutated DNA by its native, healthy sequence. Several tools for genome editing have been developed during past ten years and have been revised elsewhere [1]. Zinc Finger Nucleases (ZFNs) were the first to be developed, by fusing a zinc-finger DNA binding domain to a DNA-cleavage domain. In 2010, Sigma launched custom ready to use ZFNs to target human Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene, which had been tested on immortal cell lines for activity. On patient-specific human induced pluripotent stem cells though, these proved highly inefficient. Correction was not detected on more than one thousand clones positively transfected (personal data). Later on, in 2015, Crane and colleagues designed ZFNs to target a new site, around hundred bases upstream the previous custom one, and effectively achieved functional correction of the gene demonstrated by mRNA expression of corrected allele after directed differentiation of hIPS cells onto endoderm, anterior foregut endoderm, and up to the expression of the earliest marker of lung or thyroid epithelial cells [2]. In 2016, Bednarsky and colleagues used ZFN successfully again to functionally repair *CFTR* gene on a lung epithelial cell model by introducing a super-exon [3].

Transcription Activator-Like Effector Nucleases (TALENs) were developed after ZFNs, by fusing a TAL-effector DNA-binding domain to a nuclease. TALENs can be engineered to cut virtually any desired DNA sequence, thus increasing specificity in comparison with ZFNs. When TALENs were designed to target *CFTR* gene at same site as previous ZFNs, results showed positive correction at expected efficiencies around 10% (data published [4] and retracted after a year on the basis of an ownership report over the data we produced and which I have not been able to trace nor explain).

CRISPR-Cas9 editing system has its origin in bacterial 'immune system'. Invading viral DNA is used to create CRISPR arrays, which act as 'memory' sequences and which are used to produce RNA to target viral DNA if it attacks again. Cas9 or analogue enzyme then cuts viral DNA thus disabling viral infection. As an editing tool, the CRISPR-Cas9 system works similarly: a small RNA sequence with a guide sequence binds a specific target in the genome and cas9 protein which cuts DNA at targeted location. Schwank and colleagues used this method successfully to repair *CFTR* gene in adult-derived stem cells [5]. Lgr5+ progenitors were isolated from intestinal tissue, during surgery or after intestinal current measure on rectal suction, performed to diagnose cystic fibrosis (CF). Two years later, in 2015, Firth and colleagues used CRISPR-based genome edition to correct patient-derived human induced pluripotent (hIPS) cells [6] and demonstrated functional correction by directed differentiation of pluripotent cells to lung progenitors and up to functional lung cells on air-liquid interface cultures. To date and to the best of my knowledge, these five are the published works achieving *CFTR* correction by gene editing tools and restoring *cftr* chloride channel functionality as expected.

In summary, the achievement of restoring *CFTR* chloride channel function in target cells advances the field of stem cell/genetic therapy. Genomic edition tools allow precise and safe traceless correction of mutations. The production of differentiated cells which show normal protein function has allowed continuing research towards developing therapy. The drawback of the variable results from directed differentiation protocols has also been surpassed by the achievement in rescuing CF phenotype in adult stem cells. The future of a successful therapy is nearer as tissue-specific stem cells can be used to generate functional cells which potentially could be implanted to restore *cftr* function at least to some extent.

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