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PROCEEDINGS OF THE 17th ITALIAN CONVENTION OF FFC INVESTIGATORS IN CYSTIC FIBROSIS

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Every year, the Italian Cystic Fibrosis Research Foundation (FFC) brings together all its funded researchers from across Italy and beyond, in a Convention where results from FFC projects are shared and debated. These projects are either newly funded, ongoing or recently concluded research. The Proceedings of the 17th Italian Convention of FFC Investigators in Cystic Fibrosis report the results of the concluded research projects. Correspondence: Dr. F. Malvezzi (flaminia.malvezzi@fibrosicisticaricerca.it)

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DISEASE MODELS & PREDICTIVE TESTING

O1

A novel Full Thickness Cystic Fibrosis model on a microfluidic chip to study pathogenic mechanisms and evaluate therapeutic strategies

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Background and rationale

Cystic Fibrosis (CF) is a highly heterogeneous disease. Several airway epithelial in vitro models have been developed to better understand pathogenic mechanisms underlying CF, to investigate the patient-specific prognosis and response to therapeutics [1]. Although these models are useful, they do not recapitulate the crosstalk between epithelial cells and the connective tissue, which has important consequences on the differentiation and function/dysfunction of the epithelium [2–4].

Hypothesis and objectives

The project aimed to build up a novel 3D CF model (called Full Thickness model) featured by the presence of the lung epithelial and connective compartments. Moreover, we designed and fabricated a microfluidic device for the culture of CF models, for monitoring tissue function and for administrating drugs.

Essential methods

The normal and CF connective airway tissues (CAT) were produced by using a bottom up approach starting from the assembly of pulmonary engineered micro-tissues. In order to build up the full thickness model, normal and CF epithelial cells were seeded on the top of the normal or CF CAT and differentiated at the Air Liquid Interface. The engineered tissues were characterized by morphological, functional and molecular analysis. The microfluidic chip was designed in Autocad and fabricated in Poly Dimethyl Siloxane using a Micromilling.

Results

The CF CAT showed significant differences compared to the normal one. Specifically, CF lung fibroblasts proliferated faster and produced more elements of the extracellular matrix, featured by a higher elastic modulus. Epithelial cells developed a differentiated epithelium on the surface of the CAT and penetrated the matrix forming glandular-like structures resembling submucosal glands. The viscosity of the mucus of the CF was higher than the normal model. At the same time, the microfluidic device was developed for the culture of CF models. The chip was equipped with electrodes and an aerosol for monitoring tissue function and administrating substances in the apical side.

Conclusions

The novel 3D model well recapitulated complications occurring during CF both in the connective and epithelial compartments. For this reason, we expect it could be used to investigate the role epithelial-stroma crosstalk in CF. Moreover, the fabricated microfluidic chip could be used for the culture of CF models, for administrating drugs in the apical or serosal side of the sample and to monitor their efficacy.

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unforeseen adverse events. In this project we have integrated drug repositioning with computational studies, surface plasmon resonance (SPR) [1] and well-tried cellular models [2] to identify new CF drugs and to comprehend their mechanism of action.

Methods and results

We have prepared a new structural homology model of intact human F508del-CFTR embedded in a phospholipid bilayer and a SPR biosensor containing the same protein in a cell membrane-mimicking lipid film.

These tools, along with appropriate cell-based assays, have been firstly used to analyze a mixed library of well-known and new compounds that allowed the validation of the system and the identification of a promising molecule endowed with a F508del-binding and rescuing capacity that is higher than those of drugs already in use.

With the computational model we have then performed a virtual drug repositioning on a library of 846 drugs, identifying 10 drugs that were reduced to 4 on the basis of toxicity profile and patient compliance. These drugs will be now subjected to experimental analysis by cell-based and SPR assays for their effective capacity to bind F508del-CFTR and rescue its activity. Also, we will proceed to the virtual repositioning of a library of natural compounds.

Spin-off for research & clinical purposes

The novel computational models and biosensors will widen the study of CF drugs and made available to other research groups in the field of CF.

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GENE AND RNA EDITING

O12

Investigating CRISPR-CAS13b as a tool for the RNA editing of CFTR mRNA with premature stop codon

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Background and Rationale

Some CF patients are compound heterozygous or homozygous for nonsense mutations in the CFTR gene. Mutant CFTR gene coding for transcripts with premature termination codons (PTCs) is responsible for truncated CFTR protein and for a severe form of the disease. In a precision medicine framework the *"REPAIRv2" (RNA Editing for Programmable A to I Replacement v2*) tool, developed in the laboratory of Dr. Feng Zhang (USA), seems a good alternative to restore the full-length CFTR protein by editing its mRNA containing PTCs. This new approach is based on the possibility of targeting a deaminase enzyme (huADAR2) to a specific Adenosine, to be edited to Inosine (G analogue), on the mutant RNA by a specific guide RNA (gRNA), complementary to the target regions, and a Cas protein.

Hypothesis and objectives

We applied the new CRISPR/dCas13b based molecular tool of RNA editing (REPAIRv2) to correct the premature stop codon UGA, changing to UGG, in the H2bGFP*opal* and $CFTR^{W1282X}$ mRNAs with the purpose of recovering the full-length proteins.

Essential Methods

We designed and cloned the gRNAs needed to target the REPAIRv2 system to the Adenine to be modified. By site-directed mutagenesis we introduced a premature stop codon, W1282X, in the CFTR cDNA. Human HeLa cells expressing the H2BGFPopal mRNA, FRT cells expressing $CFTR^{W1282X}$ and IB3.1 airway epithelial human cells (CFTRΔ508/W12382X) were co-transfected with the plasmids coding for the recombinant protein dCAS13b/$ADAR2_{DD}$, and for the gRNAs. Fluorescence microscopy was used to analyse the editing results.

Results

Direct fluorescence microscopy and immunofluorescence analyses detecting the corrected proteins (H2BGFP and CFTR, respectively) suggest that the REPAIRv2 system was able, in different cell lines, to edit the H2BGFP*opal* and the $CFTRW^{1282X}$ mRNA. However, the rate of editing does not seem high. Indeed, when RNA was purified from transfected cell, retro-transcribed and amplified base correction was not detectable by standard DNA sequencing and western blot.

Conclusions

Collectively, our results indicate that the *REPAIRv2* tool is able to edit the UGA premature stop codon present in the HeLa-H2BGFP*opal* cells and in engineered FRT^{W1282X} cells harbouring the UGA PTC in the CFTR mRNA. Furthermore, the *REPAIRv2* tool worked in the IB3.1 cells suggesting its ability to edit endogenous UGA premature stop codon. Anyway, enhance the delivery of the plasmids as well increase/stabilize the target mRNA to be edited, seem necessary to improve the efficiency of *REPAIRv2*.

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O13

SpliceFix: fixing splicing defects in the CFTR gene through CRISPR/Cas9 technology

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Background/Rationale

A significant number of mutations (~13%) alter the correct splicing of the *CFTR* gene, causing the production of aberrant mRNA transcripts and non-functional protein channels. The 3242-26A>G and 3849+10kbC>T are point mutations that generate altered splicing [1–4]. The resulting mRNAs contain frameshifts in *CFTR*, producing a premature termination codons and consequent expression of a truncated non-functional CFTR protein. With this project we investigated an efficient genome editing approach to permanently correct this splicing defect [5].

Hypothesis and Objectives

The development of precise and efficient targeted nucleases has highly accelerated the progress of gene correction for genetic diseases, including Cystic Fibrosis (CF) [6]. In contrast to classical gene addition strategies, correction of the mutated *CFTR* by genome editing holds the promise to restore physiological levels of CFTR expression and function. We developed a genome editing strategy to repair