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Nasal epithelial cells as a novel diagnostic approach for Cystic Fibrosis and CFTR related-disorders

Castaldo G[1]

[1]CEINGE-Biotecnologie avanzate, Napoli (Grant No. FFC#7/2013) doi.org/92h

Background. CFTR gene sequencing enhances the detection rate of molecular analysis but it frequently identifies mutations of uncertain significance for which it is difficult to define the pathogenic role without complex functional studies that require *in vitro* expression of the mutation.

Hypothesis and objectives. Set up and validate the sampling, culture and analysis of nasal epithelial cells using a series of techniques to study the effect of mutations in a novel "*ex-vivo*" model specifically obtained from the patient bearing the novel mutation.

Methods. *Nasal brushing.* The project was approved by the Ethical Committee of the University Federico II (Naples). After nasal washings with physiological saline, nasal brushing was performed by a soft sterile interdental brush with 2.5 to 3 mm bristles (Paro-Isola) scraping along the middle portion of the inferior turbinate. *Culture of nasal cells*. The sample was cultured in serum-free bronchial epithelial cell growth medium BEGM (Clonetics). At confluence of 60%, cells were passed in new flasks after count using Invitrogen (Italy) Cell Countess. Trypan blue test was used to establish total viable cells number and the percentage of viability. *RT-PCR* was used for quantitative analysis of CFTR mRNA to test the effect of mutations that potentially impair gene expression. *RT-PCR followed by electrophoresis* was used to test the effect of splicing mutations. *Quantitative analysis of CFTR channel activity* was performed by the halide-sensitive fluorescent system.

Results. Using nasal epitheliel cells from patients, we analyzed:

- 9 mutations within the large promoter region of *CFTR* defining, for 6 of them, the pathogenic role.
- 15 mutations within the exon-intron boundaries of *CFTR* and defined the pathogenic role for 10 of them.
- the level of CFTR gating activity of 12 mutations assessing the level of the residual protein activity and correlating such data to the clinical expression.

Furthermore, combining the study on nasal epithelial cells with the *in vitro* expression analysis we defined the pathogenic role of three common complex alleles and studied genotype-phenotype correlations in 20 patients with such genotypes. Finally, we defined cut-off values for quantitative CFTR channel activity useful to discriminate between carriers and CF patients and to distinguish "severe" from "mild" mutations.

Spin-off for research & clinical purposes. Nasal epithelial cells is a scarcerly invasive and low cost approach that can be routinely used to: i) define the pathogenic role of CFTR mutations of uncertain significance; ii) analyze the gating activity of CFTR to predict the severity of the disease; iii) test the effect of drugs on the gating activity of CFTR.

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