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RIASSUNTI

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The role of functional studies in the diagnosis and treatment of cystic fibrosis: comparing the case of the G970D and G970R mutation

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Background

More than 2000 CFTR mutations have been identified so far, but only few of them are clearly defined as CF-causing based on functional studies. We present a case of a rare mutation, the G970D, that has been shown using transfected cDNA in HEK293 cells to be sensitive to Ivacaftor. However, a similar missense mutant, G970R in the same codon, was found to be sensitive to potentiators *in vitro,* but not *in vivo* due to splicing alteration. Thus, we used several basic research methodologies to evaluate if this patient was eligible for treatment with Ivacaftor.

Materials and methods

1) nasal epithelial cells (HNEC) were collected from patients to evaluate the effect of mutations on splicing by RT-PCR assay, 2) HNEC were expanded and polarized for evaluation of CFTR function by Ussing chamber system 3) the use of a minigene system was used to confirm *in vitro* the splicing pattern.

Results

Firstly, we used *in silico* tool to predict the physio-pathological effect of mutations, confirming that the G970R completely abolishes the canonical 5' splice donor site of exon 17 resulting in a likely retention of intron 17. On the contrary, the G970D predicted not to affect splicing. This prediction was confirmed by RT-PCR analysis of mRNA extracted from HNEC cells and from *in vitro* minigene assay. Finally, the functional behavior of CFTR, from HNEC bearing the G970D, was evaluated by short-circuit recordings. The cells responded to cAMP agonist with an increase in trans-epithelial current and this current was nearly doubled by stimulation with Ivacaftor. Moreover, in cells that were also incubated with VX-809 (1 μM) for 24 hours, CFTR function was significantly enhanced, with a proportional increase of both cAMP- and potentiator-dependent responses.

Conclusions

Our results show that the G970R actually disrupts the RNA splicing, thus leading to a severely altered CFTR protein. This event explains the lack of success of treatment with potentiator. In contrast, the G970D does not alter RNA splicing. The resulting G970D mutation affects the gating and trafficking of the CFTR channel that can be targeted with VX-770 and VX-809. These results represent the evidence needed to justify the treatment of the patient with these drugs. Finally, our study is an interesting example of the precision medicine approach by emphasizing the role of appropriate in vitro studies, in this case focused on RNA analysis, to fully characterize the effects of rare CFTR mutations.

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