ABSTRACT- ENGLISH VERSION

Cystic Fibrosis is a congenital, autosomal recessive chronic disease caused by CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) genetic mutations. These mutations produce thick and viscous mucus with severe damage to the pulmonary, pancreatic, hepatic, and reproductive systems. To date, more than 2000 mutations of the CFTR protein have been identified. The mutation F508del is the most frequent and has a severe phenotype.
Due to individual high variability for multiple mutations, there is not yet a definitive therapy for Cystic Fibrosis. Currently, the most used treatment is a personalized approach related to identifying CFTR mutation and using channel CFTR modulators, which can improve or partially restore the expression, function, and stability of defective CFTR protein. In this regard, four modulator drugs are available on the market, effective on patients with defined channel mutations: IVACAFTOR, ORKAMBI, SYMKEVI, and TRIKAFTA. Although these drugs can appropriately treat a great number of patients, they're not adaptable to all subjects, and also, those who are suitable for this therapy sometimes have a rather variable response. For this reason, the research in cystic fibrosis is focused on understanding the effects of drugs currently used on subjects with compatible mutations who respond in variable ways. It is also partly aimed at creating new drugs for subjects with mutations incompatible with those currently available. Nowadays, nasal potentials and respiratory and rectal organoids are the most utilized methods for monitoring therapeutic effects at a molecular level. However, their preparation could be more invasive and take time. For these reasons, over the last few years, research has focused on the possibility of using also PBMCs (Peripheral Blood Mononuclear Cells) that, in CF patients, express CFTR deficiency related to some specific dysfunctions of the immune response.
Our study aimed to identify potential leukocyte biomarkers related to therapeutic efficacy with CFTR modulators. Specifically, among the proteins modulated by IVACAFTOR therapy, matrix metalloprotease 9 (MMP9) was the principal involved. We obtained these results by analyzing both MMP9 expression and the recovery of the CFTR channel's activity in PBMCs of one patient under therapy. Indeed, the western blot analysis revealed a decrease in MMP9 expression related to the recovery of CFTR activity and an improvement of the patient's clinical parameters after therapy.
We successively analyzed MMP9 expression in PBMCs of CF patients treated with the new CFTR modulator TRIKAFTA. Even in this case, the results proved the relationship among MMP9 modulation, CFTR activity, and patients' clinical conditions.
Furthermore, to confirm that MMP9 modulation can be an excellent indicator of CFTR modulator drug efficacy, we monitored, by zymography, the MMP9 enzymatic activity directly in plasma samples of the same patients in which we had already analyzed PBMCs.

The results revealed a decrease in MMP9 enzymatic activity in the plasma of patients who responded positively to the therapies. In contrast, the enzymatic values of plasmatic MMP9 remained unchanged in patients without clinical improvements.

Finally, to obtain more information about MMP9 expression and the progression of CF disease, we studied the possible intracellular pathway, focusing on the ERK 1/2 phosphorylation and the resulting IkBα and NF-kB modulation because it is reported that NF-kB is an MMP9 transcriptional factor. Our analysis, although still preliminary, suggests that MMP9 expression in patients positively responding to the therapy is strictly under the control of the ERK 1/2 /NFKB pathway, while in the unresponsive patient, the lack of MMP9 reduction may be caused by a potential alteration in the modulation of ERK 1/2 phosphorylation.

In conclusion, MMP9 expression can be a good indicator of the efficacy of CFTR modulators. Further studies on modulating the MMP9 expression process in patients not responding to therapies will be necessary to obtain more information about the variability in patients' clinical response. This knowledge will be helpful in improving the pharmacological approach in CF.