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Sommario	Cystic fibrosis (CF), the most common autosomal recessive disease among Caucasians, is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). Among the wide spectrum of clinical and phenotypic manifestations occurring in CF, lung pathology is the main cause of morbidity and mortality. Progressive airway disease, chronic non-resolving inflammation, persistent bacterial infection are already observed in the majority of young children with CF. The prolonged airway inflammatory response induces permanent damage of CF airways leading to the loss of lung function in the majority of CF patients. In this respect, treatments with corticosteroids and ibuprofen have demonstrated potential benefits in CF patients, even though limited efficacy or the occurrence of side effects. For these reasons, the identification and the development of novel and more powerful anti-inflammatory drugs for CF airway disease remains a priority. Despite intensive research of the past few decades, the mechanisms involved in the onset of CF lung disease are not fully understood. Increasing lines of evidence highlighted the involvement of sphingolipids (SLs) in the development of CF lung pathology. SLs are cell membrane amphiphilic components that are located mainly in the external layer of the plasma membrane (PM) where they play

	important roles in the modulation of fundamental cell functions. Previous studies have demonstrated an abnormal SL metabolism in CF lung disease. In particular, increased levels of ceramide derived from sphingomyelin hydrolysis are related to the pro-inflammatory state as well as the inflammation response to bacterial infection occurring in CF lung disease. In addition, a recent study provides the evidence that ceramide derived from glycosphingolipid degradation (GSL) is involved in the inflammation response to bacterial infection of CF human epithelial bronchial cells; these data demonstrated that the pharmacological inhibition of GBA2, whose enzymatic activity produces ceramide at the cell surface, is associated with a significant reduction of the inflammatory response to P. aeruginosa infection. Moreover, GBA2 down-regulation reduces the intrinsic pro-inflammatory state typical of CF bronchial cells. On the bases of these findings, the first aim of my PhD project was to study the possible correlation between ceramide formed at the PM through the action of the PM glycohydrolases and IL-8 release and expression. The obtained results suggest that in CF bronchial epithelial cells, P. aeruginosa infection promotes the recruitment in restricted area of PM of the all glycohydrolases necessary for the complete GSL catabolism. At this site, the presence of both enzymes and their substrates allows a rapid and local change of PM architecture; this event, together with the formation of ceramide-enriched platforms might form a macromolecular complex involved in the activation of the inflammatory response. In this context, I found that GBA2 could play an important role in the development of the pro-inflammatory state typical of CF lung disease. In particular, GBA2 silencing could represent a new promising therapeutic strategy to reduce both the pro-inflammatory state and the inflammatory response to bacterial infection in CF bronchial epithelial cells. On these bases, the second objective of my PhD project was to set up a new strategy for GBA2-targeted siRNA delivery in CF epithelial bronchial cells. To this purpose, in collaboration with a group of biophysicists, I developed a lipid-based carrier to promote the transfection of genetic material. We developed two kinds of vehicles; DC-Chol/DOPE mixture assembled with siRNA (Lipoplex) and a DC-Chol/DOPE mixture with a siRNA pre-condensed with protamine (Nanoparticles). Our results showed that the Nanoparticles represented the most promising system to down-regulate GBA2 activity. Collectively, the results obtained in this study strongly support a role for GBA2 in the establishment of the pro-inflammatory state of CF. This finding provides promising bases for the use of modulators of SL metabolism as possible therapeutic strategies for CF lung inflammation.
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