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Mechanism of action of trimethylangelicin in rescuing F508del CFTR functional expression

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Background. Cystic Fibrosis Transmembrane conductance Regulator (CFTR) carrying the F508del mutation is retained in endoplasmic reticulum and fails to traffic to the cell surface where it functions as a protein kinase A (PKA)-activated chloride channel. Pharmacological correctors that rescue the trafficking of F508del CFTR could partially overcome this defect. However, the rescued F508del CFTR still displays reduced chloride permeability. Therefore, a combined administration of correctors and potentiators of the gating defect is considered to be ideal. We previously found that while a short treatment with 4,6,4'-trimethylangelicin (TMA) potentiates the cAMP/PKA-dependent activation of wild-type CFTR, a long preincubation with nanomolar concentrations of TMA significantly rescues the functional expression of F508del CFTR in primary airway cell monolayers homozygous for F508del mutation.

Objectives. The main objectives of this project were 1) identify the mechanism of action by which TMA rescues F508del CFTR activity; 2) analyze the effect of TMA treatment on actin organization and F508del CFTR stabilization on the apical membrane, 3) analyze TMA effect on cAMP/PKA compartmentalization in the membrane region.

Methods. a) The identification of CFTR region required for TMA activity was performed by Western analysis in HEK cells transfected with CFTR fragments of different length; b) the actin cytoskeleton organization and phosphoezrin localization in polarized cell monolayers were analyzed by confocal morphological analysis; c) TMA effect on cAMP and PKA compartmentalization was analyzed by FRET (Fluorescence Resonance Energy Transfer) measurements in airway cells “in vivo”.

Results. 1) TMA interacts with the MSD1 domain of CFTR 2) TMA preincubation improves the actin cytoskeleton organization and F508del CFTR stability on the apical membrane; 3) TMA rescues the cAMP/PKA compartmentalization in the membrane region of polarized CF airway cell monolayers.

Spin-off for research and clinical purposes. Altogether these results suggest that TMA may be considered a promising dual corrector/potentiator and highlight the alterations of the cellular mechanisms involved in the correctors-dependent rescue of F508del CFTR functional expression.

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