Proteins potentially involved in F508del-CFTR trafficking restoration process

P. Gomes-Alves[1,2], A.V. Coelho[2,3], F. Couto[4], P. Nogueira[5], D. Penque[1]

[1]Laboratório de Proteómica, Dept. de Genética Humana, INSA-IP, Av. Padre Cruz, 1649-016-Lisboa, Portugal
[2]Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal
[3]Departamento Química, Universidade Évora, Évora, Portugal
[4]Departamento Informática, Faculdade de Ciências-Universidade Lisboa, Lisboa, Portugal
[5]ONSA, INSA-IP, Lisboa, Portugal

Introduction

Cystic Fibrosis transmembrane conductance regulator (CFTR) is an apical membrane cAMP-regulated chloride channel defective in Cystic Fibrosis (CF). F508del-CFTR encodes an unstable and inefficiently folded protein that fails to reach the membrane. Inactivation of 4 arginine-framed tripeptides (AFTs) motifs simultaneously in CFTR sequence is an attempt to rescue the F508del-CFTR protein to be processed and functional at cell membrane (1). Using a proteomics approach we aim to unravel proteins possibly involved in the trafficking restoration process of F508del-CFTR under effect of 4RK mutation.

Methods

Total protein extracts of the cell lines BHK-wt, -F508del, -F4RK and –L (not transfected) were separated by 2D SDS-PAGE, a total of at least five gels (8-16% polyacrilamide gradient) were run per group. Gels were stained with *Blue Silver* Coomassie (2). Using the software Progenesis PG200v2006, an average gel was created for each group with a maximum number of absences allowed of 2. After protein detection and matching, normalized volume differences were compared between the four groups, only differences above 1.5 fold with a *p*-value (ANOVA)<0.05 were considered. All proteins spots differentially expressed were excised, digested with trypsin (3) and identified by MALDI-TOF or MALDI-TOF-TOF-MS.

Results

To investigate alterations in the proteome expression of BHK cells lines that are associated with heterologous expression of CFTR, BHK not-transfected (BHK-L) or stably expressing wt-, F508del- or F508del/4RK-CFTR (F4RK) were analysed by 2D/MS-proteomic approach. 780 spots were considered in the average gel of BHK-wt, 818 in BHK-F508del, 701 in BHK-F4RK and 892 in BHK-L cells. From this comparison, 71 spots were listed as differentially expressed. Comparing wt with F508del and F4RK BHK cells a total of 31 and 50 protein spots were found to be differentially expressed, respectively. However comparing BHK-F508del with -4RK cells, 47 protein spots were found differentially expressed. Based on GO terms proteins were grouped according to their molecular functions. Several of these differentially expressed proteins have been reported as directly related to CFTR, namely to the CFTR folding or ERAD (Endoplasmic Reticulum Associated Degradation), *e.g.* GRP75, GRP78/Bip, and VCP. Others although described as directly interacting with CFTR their potential role in the CFTR folding, trafficking and function are currently not assignable.

Innovative aspects

- Proteome comparison of BHK cells stably expressing the revertant (F4RK) of the most common protein mutant of CF disease, the misprocessing F508del-CFTR, with the same cell line expressing this mutant or wt-CFTR.
- Most of the differentially expressed proteins identified participate in protein folding and degradation. Some of them are chaperone and cochaperone proteins related with CFTR maturation, trafficking or degradation.
- Identified candidate proteins involved in the restoration process of the trafficking defect of F508del-CFTR can be therapeutic targets in CF.

References

(1) Chang X. *et al* (1999), Molecular Cell 4:137-42.
(2) Candiano G. *et al* (2004), Electrophoresis 25:1327-33.
(3) Miroux B. *et al* (2007), J Proteome Res 6(4):1595-602.