RNA Interference Screen to Identify Kinases That Suppress Rescue of ΔF508-CFTR*§

Agata M. Trzcińska-Danelut‡**, Anthony Chen‡**, Leo Nguyen‡, Ryan Murchie‡, Chong Jiang‡, Jason Moffat§, Lawrence Pelletier¶, and Daniela Rotin‡

Cystic Fibrosis (CF) is an autosomal recessive disorder caused by mutations in the gene encoding the Cystic fibrosis transmembrane conductance regulator (CFTR). ΔF508-CFTR, the most common disease-causing CF mutant, exhibits folding and trafficking defects and is retained in the endoplasmic reticulum, where it is targeted for proteasomal degradation. To identify signaling pathways involved in ΔF508-CFTR rescue, we screened a library of endoribonuclease-prepared short interfering RNAs (esiRNAs) that target ~750 different kinases and associated signaling proteins. We identified 20 novel suppressors of ΔF508-CFTR maturation, including the FGFR1. These were subsequently validated by measuring channel activity by the YFP halide-sensitive assay following shRNA-mediated knockdown, immunoblotting for the mature (band C) ΔF508-CFTR and measuring the amount of surface ΔF508-CFTR by ELISA. The role of FGFR signaling on ΔF508-CFTR trafficking was further elucidated by knocking down FGFRs and their downstream signaling proteins: Erk1/2, Akt, PLCγ-1, and FRS2. Interestingly, inhibition of FGFR1 with SU5402 administered to intestinal organoids (mini-guts) generated from the ileum of ΔF508-CFTR homozygous mice resulted in a robust ΔF508-CFTR rescue. Moreover, combination of SU5402 and VX-809 treatments in cells led to an additive enhancement of ΔF508-CFTR rescue, suggesting these compounds operate by different mechanisms. Chaperone array analysis on human bronchial epithelial cells harvested from ΔF508/ΔF508-CFTR transplant patients treated with SU5402 identified altered expression of several chaperones, an effect validated by their overexpression or knockdown experiments. We propose that FGFR signaling regulates specific chaperones that control ΔF508-CFTR maturation, and suggest that FGFRs may serve as important targets for therapeutic intervention for the treatment of CF.

© 2015 by The American Society for Biochemistry and Molecular Biology, Inc.

From the ‡Program in Cell Biology, The Hospital for Sick Children, Toronto, and Biochemistry Department, University of Toronto; PGCRL, 19–9715, 686 Bay St., Toronto, Ont., Canada, M5G 0A4 §CCBR, University of Toronto; ¶The Samuel Lunenfeld Research Institute, University of Toronto

Received November 7, 2014, and in revised form, March 24, 2015

Published, MCP Papers in Press, March 29, 2015, DOI 10.1074/mcp.M114.046375

Author contributions: A.M.T. and D.R. designed research; A.M.T., A.C., L.N., R.M., and C.J. performed research; C.J., J.M., and L.P. contributed new reagents or analytic tools; A.M.T., A.C., L.N., R.M., C.J., and D.R. analyzed data; A.M.T. and D.R. wrote the paper.

CF. Molecular & Cellular Proteomics 14: 10.1074/mcp. M114.046375, 1569–1583, 2015.

Cystic fibrosis (CF) is a pleiotropic disease caused by an abnormal ion transport in the secretory epithelia lining the tubular organs of the body such as lungs, intestines, pancreas, liver, and male reproductive tract. In the airways of CF patients, reduced Cl− and bicarbonate secretion caused by lack of functional Cystic fibrosis transmembrane conductance regulator (CFTR) on the apical surface, and hyper-absorption of Na+ because of elevated activity of ENaC (1), lead to a dehydration of the airway surface liquid (ASL). This reduces the viscosity of the mucus layer and the deposited layer of thickened mucus creates an environment that promotes bacterial colonization, which eventually leads to chronic infection of the lungs and death (2, 3).

CFTR is a transmembrane protein that functions as a cAMP-regulated, ATP-dependent Cl− channel that also allows passage of bicarbonate through its pore (4, 5). It also possesses ATPase activity important for Cl− conductance (6, 7). The CFTR structure is predicted to consist of five domains: two membrane spanning domains (MSD1, MSD2), each composed of six putative transmembrane helices, two nucleotide binding domains (NBD1, NBD2), and a unique regulatory (R) region (8).

More than 1900 CFTR mutations have been identified to date (www.genet.sickkids.on.ca/cftr). The most common mutation is a deletion of phenylalanine at position 508 (ΔF508 or ΔF508-CFTR) in NBD1 (9). The ΔF508 mutation causes severe defects in the processing and function of CFTR. The protein exhibits impaired trafficking from the endoplasmic reticulum (ER) to the plasma membrane (PM), impaired intramolecular interactions between NBD1 and the transmembrane domain, and cell surface instability (10–15). Nevertheless, the ΔF508 defect can be corrected, because treating cells expressing ΔF508-CFTR with low temperature or chemical chaperones (e.g. glycerol) can restore some surface expression of the mutant (11, 16).

1 The abbreviations used are: CF, cystic fibrosis; CFTR, Cystic fibrosis transmembrane conductance regulator; RTK, receptor tyrosine kinase; Hsp, heat shock protein.
Numerous small molecules that can at least partially correct (or potentiate) the ΔF508-CFTR defect have been identified to date (17–27), and some were already tested in clinical trials (e.g. sildenafil, VX-809/Lumacaftor), or have made it to the clinic (VF770/Kalydeco/Ivacaftor) (http://www.cff.org/research/DrugDevelopmentPipeline/). However, the need to identify new ΔF508-CFTR correctors remains immense as the most promising corrector, VX-809, has proven ineffective in alleviating lung disease of CF patients when administered alone (27). Thus, our group developed a high-content technology aimed at identifying proteins and small molecules that correct the trafficking and functional defects of ΔF508-CFTR (28). We successfully used this approach to carry out three separate high-content screens: a protein overexpression screen (28), a small-molecule kinase inhibitor screen (29) and a kinome RNAi interference (RNAi) screen, described here.

EXPERIMENTAL PROCEDURES

Media and Reagents—Dublecco’s Modified Eagle’s Medium (DMEM), Dulbecco’s Phosphate Buffered Saline (pH 7.4), Fetal Bovine Serum (FBS), trypsin, G418, blasticidin, and zeocin were obtained from Invitrogen (Carlsbad, CA). The mouse M3A7 anti-CFTR monoclonal antibody was purchased from Millipore, Temecula, CA, the mouse HA.11 (16B12) monoclonal antibody was from Covance (San Diego, CA), the rabbit polyclonal antivinculin antibody was from Abcam (Cambridge, UK), and SuperSignal West Femto Maximum Sensitivity kit was from Pierce (Rockford, IL). The High Capacity cDNA Reverse Transcription kit was obtained from Applied Biosystems (Foster City, CA), the Platinum® SYBR® Green qPCR SuperMix-UDG was from Invitrogen, and the SA-HRP was from eBioscience. The kinome RNA interference (esiRNA) library was obtained from Dr. Laurence Pelletier (The Samuel Lunenfeld Research Institute - see below). shRNA clones were from the RNAi Consortium (TRC) (30) and SIDNET/SPARC BioCentre, The Hospital for Sick Children (pGIPZ). For the overexpressed chaperones, the entry clones compatible with Gateway® system were obtained from SIDNET/SPARC BioCentre and PlasmID (The Dana-Farber/Harvard Cancer Center DNA Resource Core), and were subsequently cloned into the destination vector, pcDNA3.1(eYFP H148Q/I152L). All constructs were sequence verified for accurate coding.

Cloning and Cell Line Generation—The triple hemagglutinin (3HA) tag was cloned by PCR based on the sequence used in BHK ΔF508-CFTR 3HA cell line (22), and inserted after Asn at position 901 (N901) in the fourth external loop of CFTR. The full length CFTR bearing 3HA tag (wild type or ΔF508) was subsequently cloned into the pLVE/zeo vector. The plasmids were then transfected into the HEK293 MSR GripTite using calcium phosphate precipitation. The transfected cells were seeded at different concentrations to isolate individual colonies under selection with 100 μg/ml zeocin. Individual clones were picked, expanded and CFTR or ΔF508-CFTR expression verified by immunoblotting (supplemental Fig. S1).

Cellomics YFP Halide-exchange Assay—

Kinome esiRNA Library—The kinome cassette of a genome-scale library of endoribonuclease-prepared short interfering (esi)RNAs (30) contains dsRNA pools that target 759 different kinases and associating signaling proteins (supplemental Table S1).

esiRNA Screen and Drug Combination Testing Using the Cellomics YFP Halide-Exchange Assay—HEK293 MSR GripTite (293MSR-GT) cells stably coexpressing eYFP (H148Q/I152L) and ΔF508-CFTR proteins (29) were cultured in DMEM medium supplemented with 10% FBS, 1× Nonessential Amino Acids, 0.6 mg/ml G418, 10 μg/ml blasticidin, and 50 μg/ml zeocin, at 37 °C, 5% CO2 in humidified atmosphere.

The Cellomics halide-exchange assay was performed as described previously (29). Briefly, 5 × 10^4 ΔF508-CFTR cells (i.e. 293MSR-GT cells stably coexpressing eYFP (H148Q/I152L) and ΔF508-CFTR) per well were seeded in 96-well plates. The next day the cells were transfected with esiRNA duplexes from the library (final concentration 40 nm), luciferase (nonsilencing control), EG5 (transfection control), or AHA1 esiRNA (assay control), using Lipofectamine 2000. Medium was changed 6 h after transfection, and the cells were placed at 37 °C, 5% CO2 for 72 h. The 96-well transfection protocol was optimized using EG5 (KIF11) esiRNA as a transfection control. The transfection was considered successful if more than 80% of ΔF508-CFTR cells exhibited round-shape phenotype 72 h post-transfection (see “Results”). After 72 h of incubation, the medium was replaced with 152 μl of chloride solution (137 mM NaCl, 2.7 mM KCl, 0.7 mM CaCl2, 1.1 mM MgCl2, 1.5 mM KH2PO4, 8.1 mM Na2HPO4, pH 7.1), in the absence or presence of FIG (25 μM Forskolin, 45 μM IBMX, 50 μM Genistein), at 37 °C. After 20-min incubation, 92 μl of iodide buffer (137 mM NaCl, 2.7 mM KCl, 0.7 mM CaCl2, 1.1 mM MgCl2, 1.5 mM KH2PO4, 8.1 mM Na2HPO4, pH 7.1) was added (final I– concentration 52 mM). Using the Cellomics KineticScan KSR Reader (Thermo Fisher) and a modified Target Activation algorithm, objects (individual cells or sometimes clusters of cells) were defined by eYFP(H148Q/I152L) fluorescence intensity, and the fluorescence quenching over 24-s time course at 30 °C, 5% CO2 was recorded. Well contents were scanned between 70 and 300 objects per field (single field per well). Genes that displayed a difference in the YFP fluorescence intensity (between FIG-stimulated sample and nonsilencing control) lower than 0.09 were rejected after the first two runs of the screen. This cut-off value equaled three times the standard deviation from the mean value of the control (AHA1). The rest of the esiRNA duplexes (56 genes) were subjected to the third run of the screen. Twenty top hits of the screen were subjected to further validation of ΔF508-CFTR rescue by functional assay (Cellomics ArrayScan VTI platform) immunoblotting and ELISA following shRNA-mediated knockdown.

For drug combination testing, 8 × 10^4 ΔF508-CFTR cells (i.e. 293MSR-GT cells stably coexpressing eYFP (H148Q/I152L) and ΔF508-CFTR) per well were seeded in 96-wells plates. The next day, the cells were treated with either SU5402 + VX809 or AZD4547 + VX809 with concentration ranging from 1 μM to 10 μM. The cells were then incubated at 37 °C, 5% CO2 for 48 h, and analyzed by the Cellomics halide-exchange assay, as above.

Validation of the esiRNA Screen—

Cellomics Analysis Following shRNA Knockdown—Prior to the Cellomics halide-exchange assay ΔF508-CFTR cells (stably expressing eYFP (H148Q/I152L)) were transfected with shRNA constructs targeting the identified genes or luciferase (nonsilencing control), using Lipofectamine 2000, according to the manufacturer’s instructions. Medium was changed 6 h after transfection and ΔF508-CFTR cells were placed at 37 °C, 5% CO2. Forty-eight hours after transfection the cells were incubated with media containing puromycin (5 μg/ml, 3 days). Cellomics halide-exchange assay was performed as described above, using Cellomics ArrayScan VTI platform (data from three fields per well). A total number of 133 shRNA clones were screened (multiple shRNA clones per gene) (supplemental Table S2). Knockdown efficiency was validated by two-step RT-qPCR as described previously (29). Briefly, total RNA was isolated using the RNeasy 96 kit (Qiagen, Venlo, Netherlands), and cDNA was prepared using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems). Real-time PCR reactions were performed using Platinum® SYBR® Green qPCR SuperMix-UDG (Invitrogen) and CFX96 Real-Time System (BioRad). Primers were obtained from Integrated DNA Technologies. For
was placed against the injected cheek, for 4 min. Isoprenaline (10 mM, subcutaneously injected into the right cheek to block potential cho-

stimulated using 25 μM Forskolin, 25 μM IBMX and 50 μM Genistein (FIG), and after the indicated time (min) inhibited using 15 μM GlyH-101 (Gly). Data were recorded and analyzed using Analyzer 2.1.3. Knockdown efficiency was validated by two-step RT-qPCR, as described above.

Salivary Secretion Assay (SSA)—The salivary secretion assay, described previously (31), was modified as follows. Male ΔF508 mice (C57BL/6J FVB) on a 129/FVB background and their wild-type littermates (kindly provided by Dr. C. Bear) of 9–12 weeks were intra-peritoneally injected with DMSO or SU5402 (dissolved in DMSO at the concentration of ΔF508-CFTR mice and wild-type littermates were generated and maintained in culture, as described (32). For forskolin-induced swelling (FIS) experiments, organoids were seeded in 24-well tissue culture plates, pretreated with kinase inhibitors (SU5402, 10 μM) and/or VX-809 (3 μM), and stimulated with 5 mM forskolin, as outlined in (33). FIS was observed by brightfield live-cell microscopy with an automated xy-stage (Nikon TE-2000 with Solent Scientific enclosure, 20×).

Chaperone Array Screen—Human Bronchial Epithelial (HBE) cells from ΔF508/ΔF508-CFTR transplant patients (P2 cells) were obtained from the University of Iowa Cell Culture Facility and grown on collagen-coated permeable millipore inserts. The cells were treated with DMSO (control), 1 μM or 10 μM SU5402 for 48 h prior to RNA extraction. Total RNA was extracted using the PureLink RNA Mini Kit (Invitrogen) and cDNA was synthesized from 1 μg of mRNA using the High capacity cDNA reverse transcription kit (Applied Bioscience) according to the manufacturer’s instructions. Array analysis was performed using the RT² Profiler™ PCR Array Human Heat Shock Proteins & Chaperones kit (Qiagen). mRNA expression levels were determined relative to actin, GAPDH and B2M using the ΔΔCt method. Changes in chaperone expression level relative to DMSO control were determined using the ΔΔCt method. The chaperone array experiment was performed three times and average values are shown in a heat map.

Validation of the Chaperone Array Hits—ΔF508/ΔF508-CFTR 3HA cells per well were seeded in a 6-well plate format. The next day the cells were transfected with the clones for the analyzed chaperone genes (shRNA or overexpression) or luciferase control, using PolyJet™ DNA In Vitro Transfection Reagent according to the manufacturer’s instructions. Forty-eight hours post-transfection, the cells that were transfected with shRNA were further incubated with media containing puromycin (5 μg/ml, 3 days). The cells that were transfected with the chaperone overexpression clones were biotinylated, and ELISA was performed as described above.

HSF1 Experiments—The pcDNA3.1(eYFP H148Q/I152L) plasmid containing the wild-type HSF1 was used to construct a constitutively active mutant of HSF1 (34) using site directed mutagenesis consisting of one-step PCR with two overlapping internal primers at the mutagenic site. The internal primers used were 5’GAA-

Identification of Kinases and Associated Signaling Proteins That Suppress Rescue of ΔF508-CFTR—Delineation of pathways and proteins that prevent rescue of ΔF508-CFTR is important for the identification of drugs that target these pathways. Our group previously developed a high-content functional screen to identify ΔF508-CFTR correctors (and poten-
tiators) in multiple individual cells simultaneously, using Cellomics KineticScan KSR and ArrayScan VTI platforms (28, 29). The kinome esiRNA screen presented in this study complements the small-molecule kinase inhibitor screen described previously (29).

We screened a library of esiRNA duplexes targeting 759 different kinases and associated proteins (supplemental Table S1). Because esiRNA transfection efficiency is critical to performing this screen, and the esiRNA molecules do not carry a selection marker, the 96-well transfection protocol was optimized using EG5 (KIF11) esiRNA as a transfection control added to parallel wells on the same plate. Knockdown of EG5, a kinesin-related motor protein, leads to mitotic arrest and rounded-up phenotype of the cells. The transfection was considered successful if more than 80% of ΔF508-CFTR cells exhibited round-shape phenotype 72 h post-transfection.

In the Cellomics halide-exchange assay the fluorescence quenching of YFP, corresponding to Cl⁻/I⁻ exchange via ΔF508-CFTR, was quantified in cells stably expressing ΔF508-CFTR and transfected with esiRNA duplexes from the library. Fig. 1 depicts several representative “hit” suppressors that when knocked-down exhibited various degrees of correction of the ΔF508-CFTR defect. The complete list of the hits, defined as those exhibiting a difference in average fluorescence intensity ΔFIavg between Forskolin-IBMX-Genistein (FIG)-stimulated sample and FIG-stimulated nonsilencing control of at least 9%, is provided in Table I. The cut-off value of 9% (0.09) was chosen as it equals three times the standard deviation from the mean value of the control (AHA1). Interestingly, the “hit” list reveals novel suppressors involved in Ras/Raf/MEK/Erk and PI3K/Akt signaling pathways, along with the kinases previously identified by our small-molecule kinome

![Fig. 1. Representative hits of the kinome esiRNA screen.](image-url)
inhibitor screen (e.g. FGFR1, Raf) (29), which further validates our approach.

As both the transfection and knockdown efficiency can influence the level of rescue observed in our esiRNA screen, we further validated the hits (and possible off-target effects) by carrying out an independent high-content screen, using shRNA.

**Validation of the Hits**—To validate the effect of knockdown of the suppressor genes on \( \Delta F_{\text{avg}} \) of \( \Delta F \) between FIG-stimulated and nonsilencing control of at least 9%, we tested the top “hits” with shRNA. For this we screened 133 shRNA clones targeting 20 suppressors identified in our kinome esiRNA screen (supplemental Table S2).

\( \Delta F \) of \( \Delta F_{\text{avg}} \) between FIG-stimulated and nonsilencing control of at least 9% of the analyzed suppressor genes luciferase (nonsilencing control) were analyzed in the Cellomics halide-exchange assay. In parallel, qPCR was performed to determine the knockdown efficiency of these constructs. Although the shRNA clones yielded varied degrees of knockdown, most of them resulted in more than 50% knockdown of target genes (supplemental Table S2), and in general, the degree of \( \Delta F_{\text{avg}} \) rescue correlated with knockdown efficiency. In the case of MET and BRAF genes, cell death was observed upon knockdown higher than 60–70% and, therefore, shRNA clones that resulted in the best rescue exhibited knockdown of 30% (B-Raf)–60% (MET).

The results of the shRNA screen corroborate the esiRNA hits (Fig. 2 and Table II). Out of 20 analyzed genes, 14, when knocked-down by shRNA, produced reproducible rescue of \( \Delta F_{\text{avg}} \) of 12–30%. These included genes encoding the receptor Tyr kinases FGFR1 and MET, as well as B-Raf and the MAPK kinase kinase MAP3K13. Examples of other hits are the receptor interacting protein kinase RIPK4, the sedoheptulokinase SHPK, the cyclin-dependent kinase CDK10, the suppressor of cytokine signaling SOCS1, and the PKA regulatory subunit PRKAR2B. The knockdown of six genes, PANK1, NEK10, CLK3, DTYMK, ERN1, and CAMK2B, led to a lower degree of rescue (\( \Delta F_{\text{avg}} < 10\% \)) (Table II).

To further validate the hits, we analyzed maturation of \( \Delta F \) at the plasma membrane, we employed an ELISA assay. For this, we generated a new stable HEK293 GT cell line that expresses \( \Delta F \) with a triple HA tag at the ectodomain (supplemental Fig. S1A). The ELISA experiments revealed a prominent increase (approx. 50%) in the amount of surface \( \Delta F \) following knockdown of FGFR1 relative to control knockdown (Fig. 3). Other robust inhibitors (increase in \( \Delta F_{\text{avg}} \) surface expression by at least 20%) were SHPK, BRAF, CDK10, IPMK, DUSP22, PANK4, SOCS1, and NEK10. The knockdown of six genes (MET, MAP3K13, RPS6KC1, PRKAR2B, PCK2, CAMK2B, DTYMK, CLK3, and PANK1) resulted in a lower degree of rescue (10–20%, Fig. 3).

### Table I

**Results of the esiRNA screen. The top 20 hit genes that displayed a difference in average fluorescence intensity \( \Delta F_{\text{avg}} \) (between FIG-stimulated sample and nonsilencing control) of at least 9%**

| Gene name | Protein name Accession No. | Rescue (%) |
|-----------|---------------------------|------------|
| RIPK4     | Receptor-interacting serine/threonine-protein kinase 4 P57078 | 22 |
| SHPK      | Sedoheptulokinase Q9UHJ6 | 20 |
| MAP3K13   | Mitogen-activated protein kinase kinase kinase 13 O43283 | 19 |
| FGFR1     | Fibroblast growth factor receptor 1 P11362 | 16 |
| CDK10     | Cyclin-dependent kinase 10 Q15131 | 16 |
| RPS6KC1   | Ribosomal protein S6 kinase delta-1 Q96538 | 16 |
| PANK4     | Pantothenate kinase 4 Q9NV7E | 14 |
| DTYMK     | Thymidylylate kinase P23919 | 14 |
| ERN1      | Serine/threonine-protein kinase/endoribonuclease IRE1 O75460 | 14 |
| BRAF      | Serine/threonine-protein kinase B-raf P15056 | 13 |
| DUSP22    | Dual specificity protein phosphatase 22 Q9NRW4 | 13 |
| IPMK      | Inositol polyphosphate multikinase Q8NFU5 | 13 |
| PCK2      | Phosphoenolpyruvate carboxylase Q16822 | 13 |
| CLK3      | Dual specificity protein kinase CLK3 P49761 | 13 |
| MET       | Tyrosine-protein kinase Met P08581 | 12 |
| CAMK2B    | Calcium/calmodulin-dependent protein kinase type II subunit beta Q13554 | 12 |
| SOCS1     | Suppressor of cytokine signaling 1 Q15524 | 11 |
| NEK10     | Serine/threonine-protein kinase Nek10 Q6ZWH5 | 11 |
| PRKAR2B   | cAMP-dependent protein kinase type II-beta regulatory subunit P31323 | 9 |
| PANK1     | Pantothenate kinase 1 Q8TE04 | 9 |
In order to measure ΔF508-CFTR channel activity by short-circuit current of our hits, 10 human-to-canine compatible shRNA clones (CDK10, PANK1, PANK4, RPS6KC1, DUSP22, SOCS1, FGFR1, CLK3, NEK10, BRAF, PCK2, and IPMK) were transduced (via lentiviral infection) into MDCK cells stably expressing ΔF508-CFTR (28, 29). Knockdown efficiency was measured by qPCR. PANK1, PANK4, and NEK10 genes showed no expression in the MDCK cells, and the knockdown level of three others (BRAF, PCK2, and SOCS1) was negligible. The remaining genes (CDK10, RPS6KC1, DUSP22, FGFR1, CLK3, and IPMK) exhibited knockdown level of 39–86% (supplemental Table S2), and were subjected to short-circuit current (\(I_{sc}\)) analysis in Ussing chambers. Three of the analyzed genes, RPS6KC1, IPMK, and CLK3, partially restored the ΔF508-CFTR function, as shown by an increase in short-circuit current (21–50%) (supplemental Fig. S2). As MDCK cells exhibited an increased sensitivity toward knockdown of CDK10, DUSP22, and FGFR1 (changes in proliferation rate and/or cell morphology), we were unable to assess ΔF508-CFTR chloride channel activity in the cells that expressed shRNAs for these genes.

FGFR Signaling Plays an Important Role in the Maturation of ΔF508-CFTR—In our previous study (29) we proposed that FGFRs, and possibly other Receptor Tyrosine Kinases (RTKs), suppress maturation of the ΔF508 mutant. To further explore the role of FGFRs in the folding and/or trafficking of ΔF508-CFTR, we knocked down FGFR1, 2, and 3, and their select downstream signaling proteins. ΔF508-CFTR cells were transfected with shRNA for FGFRs, Erk1 and Erk2, Akt, PLCγ-1, FRS2α, or luciferase (nonsilencing control). We then performed the Cellomics halide-exchange assay and immunoblotting analyses. The effect of the knockdown on ΔF508-CFTR maturation and function is shown in Fig. 4. Knockdown of the analyzed genes (i.e. FGFRs, Erk1, Erk2,
Validation of hits with shRNA using the halide-exchange assay

| Gene name | Validation by functional assay (Cellomics) | Knockdown level (%) |
|-----------|--------------------------------------------|---------------------|
| FGFR1     | 10                                         | 0.30                | 94                  |
| RIPK4     | 2                                          | 0.22                | 26                  |
| MET       | 17                                         | 0.16                | 61                  |
| SHPK      | 3                                          | 0.15                | 52                  |
| MAP3K13   | 4                                          | 0.15                | 65                  |
| BRAF      | 7                                          | 0.15                | 34                  |
| DUSP22    | 7                                          | 0.15                | 71                  |
| CDK10     | 6                                          | 0.14                | 87                  |
| IPMK      | 10                                         | 0.13                | 63                  |
| RPS6KC1   | 2                                          | 0.13                | 96                  |
| PRKAR2B   | 2                                          | 0.12                | 40                  |
| PANK4     | 15                                         | 0.12                | 80                  |
| SOCS1     | 5                                          | 0.12                | N/A                 |
| PCK2      | 4                                          | 0.12                | 78                  |
| CAMK2B    | 3                                          | 0.09                | 85                  |
| DTYMK     | 10                                         | 0.09                | 88                  |
| ERN1      | 1                                          | 0.08                | 79                  |
| CLK3      | 8                                          | 0.08                | 64                  |
| NEK10     | 10                                         | 0.08                | 84                  |
| PANK1     | 7                                          | 0.06                | 79                  |

Additive Effect of FGFR Inhibitors and VX-809 on Rescue of ΔF508-CFTR—Clinical tests of VX-809 administered alone to ΔF508-CFTR patients have not yielded a significant improvement in lung function of these patients (27), although its combination with VX-770 yielded a small improvement...
(http://investors.vrtx.com/releasedetail.cfm?ReleaseID=856185); however, VX-770 was recently shown to reduce functional expression of VX-809 (37, 38). Thus, we tested the effect of SU5402 (or another FGFR inhibitor, AZD4547) together with VX-809 using the Cellomics assay. Fig. 8 and supplemental Table S4 show that at 1 to 10 μM of both SU5402 (or AZD4547) and VX-809, there was an additive effect on rescue of ΔF508-CFTR, suggesting these compounds act by different mechanisms, an observation also supported by analysis of rescue of ΔF508-CFTR in mouse intestinal organoids (Fig. S5D).
FIG. 5. Rescue of ΔF508-CFTR in intestinal organoids from ΔF508/ΔF508 mice. A–D, Rescue of ΔF508-CFTR with SU5402 analyzed in intestinal organoids from ΔF508/ΔF508 mice by organoids swelling: A and B, Intestinal organoids derived from crypts isolated from the terminal ileum of ΔF508 mice (and WT littermate controls) were treated (at 37 °C) with 5 μM forskolin for 50 min to activate CFTR, leading to swelling via chloride efflux and lumenal fluid accumulation by WT-CFTR but not ΔF508-CFTR. Scale bars = 50 μm. C, ΔF508 organoids do not exhibit increased surface area following forskolin treatment, but swelling can be rescued at low temp: As in B, except one batch of ΔF508 organoids were preincubated at 27 °C for 24 h prior to assay, revealing temp. rescue. D, Treatment with SU5402 or VX-809 partially rescued swelling in ΔF508-CFTR organoids. ΔF508 organoids were pretreated with VX-809 (3 μM; green) or SU-5402 (10 μM; red), administered to the organoid culture media. Treatment with either compound (at 37 °C) partially rescued CFTR-mediated swelling in the ΔF508 organoids, a rescue augmented by combining both SU5402 and VX-809. Error bars for panels B–D represent mean ± S.D. n = 20–30 organoids per treatment. Forsk: Forskolin.
DISCUSSION

Our previous identification, using small-molecule kinase inhibitors, of several signaling cascades (e.g. Ras/Raf/MEK/Erk, Wnt/GSK-3β, PI3K/Akt/mTOR, TAK1/p38) (29) that regulate ΔF508-CFTR trafficking and maturation prompted us to perform a more systematic screen for all human kinases and select associated signaling proteins that may regulate ΔF508-CFTR rescue. We therefore performed an esiRNA human

Fig. 6. Chaperone expression analysis following FGFR inhibition by SU5402. A, Response (ISC) of ΔF508/ΔF508-CFTR HBE cells obtained from two separate patients to SU5402 analyzed by Ussing chambers, highlighting the response of the Responder and Poor responder to 48 h treatment with 1 μM or 10 μM SU5402. CFTR currents were stimulated with FIG (25 μM Forskolin, 25 μM IBMX, and 50 μM Genistein) and after the indicated time inhibited using 15 μM GlyH-101 (Gly). Inset: summary of three separate experiments (mean ± S.E.). B, Heat map of chaperone expression profile following 48 h treatment of ΔF508/ΔF508-CFTR HBE cells (from the Responder and Poor responder) with 1 μM or 10 μM SU5402, as indicated. Data are average of three separate arrays (normalized to DMSO control).
kinome screen using our Cellomics high-content assay to search for suppressors that block maturation of ΔF508-CFTR and hence, when knocked-down, lead to rescue of this mutant. The top hits (20 genes) were validated with another RNAi technology, shRNA, to ensure that the rescue observed in the original esiRNA screen was not caused by off-target effects. In parallel, shRNA constructs were used to test the effect of knockdown of these genes on ΔF508-CFTR maturation and surface expression by immunoblotting and ELISA, respectively. The knockdown of several of the analyzed genes (e.g. FGFR1, SHPK, MAP3K13, DUSP22, CDK10, IPMK, and PANK4) led to a substantial rescue of ΔF508-CFTR activity in the halide-exchange assay, and a corresponding robust increase in cell surface amount of ΔF508-CFTR.

The results of both RNAi screens (esiRNA and shRNA) lend further support to the results obtained in our small-molecule kinase inhibitor screen (29). Several of the hits are kinases that are either direct targets, or are involved in the signaling cascades by the kinase inhibitors discovered in our earlier compound screen. For example, FGFR1 was the target of several ΔF508-CFTR correctors that were previously identified in our kinase inhibitor screen (e.g. SU5402, SU6668, PD173074) (29), and in accord, its knockdown here also led to a substantial rescue of this CFTR mutant.

In this study, we show that knockdown of FGF receptors (FGFR 1, 2, and 3), or their intermediate signaling proteins such as PLC-γ1, Erk1/2, Akt, and FRS2α, can stimulate rescue of ΔF508-CFTR trafficking. Furthermore, our results suggest that FGFR1 inhibitors (e.g. SU5402) may prove useful in treatment of CF: The treatment of the ΔF508-CFTR mice with SU5402 led to the increase of the isoprenaline-stimulated salivary secretion (~10% of the wild-type CFTR mice level), which corresponds to a partial correction of the ΔF508-CFTR defect, and a strong rescue of ΔF508-CFTR with SU5402 was observed in intestinal organoids harvested from these mice. Given the importance of even a partial (10–25%) rescue of

Fig. 7. Validation of chaperone hits.
ELISA analysis of cell surface expression of ΔF508-CFTR (relative to luciferase control) expressed in 293-GT cells following, A, overexpression of the top chaperones that exhibited elevated expression following SU5402 treatment in the heat map (Fig. 6), or B, shRNA-mediated knockdown of chaperones that exhibited reduced expression in the heat map following SU5402 treatment. The extent of knockdown (two clones per gene) is shown in Supplemental Table S3. Data in A and B are mean ± S.E., n = three independent experiments.
Kinome RNAi Screen for Rescue of ΔF508-CFTR

ΔF508-CFTR for improvement in CF patients’ health (39, 40), our findings could have significant clinical implications. Moreover, modifications of drug dose, scheduling, mode of administration, and the development of SU5402 more potent analogs, may further improve the efficacy of this (or related) compound for the treatment of CF. In addition, the additive effect of SU5402 and VX-809 on ΔF508-CFTR rescue suggests that these compounds act on different cellular targets and hence drug combination may be useful for future CF treatment.

FGFRs are known to activate the Ras/Raf/MEK/Erk and PI3K/Akt pathways (41–43). In addition to FGFRs, among the RTKs identified by our group was HGF Receptor (Met), another potent regulator of Ras/Raf/MEK/Erk and PI3K/Akt pathways. Nevertheless, how RTKs suppress the rescue of ΔF508 mutant is still unknown, but likely involves inhibition of specific chaperones that normally promote folding and maturation of CFTR or its ΔF508 mutant (e.g. Hsp90 complex), or stimulate inhibitory chaperones/cochaperones and their partner E3 ligases (e.g. Hsp70/CHIP or RMA1 complexes) (28, 44–50). In agreement, Erk1 and Erk2 were shown to inhibit Heat Shock Factor 1 (HSF1) activity and thus to suppress the subsequent expression of heat shock proteins (hsp) (51–55). HSF1 was shown to induce the expression of numerous hsp, for example hsp70 (HSPA1A), hsp60 (HSPD1), hsp40 (DNAJ), hsp27(HSPB2), and CRYAB (HSPB5) (hsp20 family) (56–60), as well as HspE1, HspH1, SERPINH1, and Hsp90AB1 (61); several of these (and other) HSF1 effectors were identified in our current array analysis. In support, hsp70, hspA4 and CRYAB were previously shown by us and others to enhance ΔF508-CFTR maturation (28, 62), as did a constitutively active HSF1. Furthermore, the positive role of HSF1 in the correction of various conformational disease models, including cystic fibrosis, was shown recently (63). Other kinases discovered by the esiRNA screen that are involved in MAPK signaling are B-Raf, MAP3K13, and CaMKIIβ. B-Raf acts downstream of Ras and activates Erk1/2 via MEK kinases (64, 65). MAP3K13 plays a role in the activation of JNK and NFκB (66, 67), and CaMKIIβ (Ca²⁺/calmodulin-dependent protein kinase II beta) was shown to activate TAK1, which in turn leads to phosphorylation of hsp27 (68–70). The phosphorylation of hsp27 promotes switching off the hsp27 folding activity in favor of targeting the substrates for degradation (71–73). In support, hsp27 was identified as a component of the CFTR interactome (48), and has recently been reported to target ΔF508-CFTR for degradation via a SUMO-dependent pathway (74).

The results of our RNAi screen also support a negative role of the NFκB signaling in the maturation of ΔF508-CFTR. We identified RIPK4, a member of the Receptor Interacting Protein Kinase family, which is known to activate NFκB (75). The elevated NFκB-mediated IL8 signaling is one of the main contributors to the chronic hyper-inflammation in the CF lung (76–78). However, how the NFκB pathway inhibits maturation of ΔF508-CFTR needs to be elucidated.
We also identified SOCS1 (Suppressor of Cytokine Signaling 1) as an inhibitor of ΔF508-CFTR rescue. SOCS proteins block STAT phosphorylation by inhibiting Janus kinases (JAKs) activity or competing with STATs for binding sites on cytokine receptors (79–81). In support, we previously demonstrated that overexpression of STAT1 promotes the rescue of ΔF508-CFTR (28).

Another interesting gene identified in our esiRNA screen is PRKAR2B, which encodes the RIIβ regulatory subunit of cAMP-dependent kinase PKA. Stimulation by PKA is known to promote CFTR channel activity, enhance CFTR trafficking, and to decrease CFTR endocytosis from the plasma membrane (82, 83). It was shown that the cAMP-binding domain B of RIIβ subunit inhibits the PKA holoenzyme activation (84). Therefore, the knockdown of this inhibitory subunit could lead to an increase of PKA activity, which in turn promotes ΔF508-CFTR rescue.

Our screen also identified ERN1 (Serine/threonine-protein kinase/endoribonuclease IRE1 or Inositol-requiring protein 1) and IPMK (Inositol Polyphosphate Multikinase) as suppressors of ΔF508-CFTR rescue. ERN1/IRE1 signaling is known for its role in the unfolded protein response in the ER lumen and was reported to reduce the level of misfolded CFTR (85). IPMK is a PI3-kinase that acts as a molecular switch for Akt, inhibiting or stimulating PI3K/Akt signaling (86). PI3K/Akt cascade, along with Ras/Raf/MEK/ERK, is known to affect expression and function of numerous chaperones (87). However, the exact role of IPMK in CFTR maturation and trafficking requires further investigation.

In summary, this study has identified several novel suppressors of ΔF508-CFTR rescue. These suppressors modulate or belong to important cellular signaling pathways such as Ras/Raf/MEK/ERK, PI3K/Akt, p38, and NFκB. The effect of receptor Tyr kinases (especially FGFRs) on ΔF508-CFTR maturation and chaperone expression in ΔF508/ΔF508-CFTR HBE was further elucidated. Thus, identifying proteins and their respective pathways that control rescue of ΔF508-CFTR is a powerful approach to identify drugs that target these same proteins/pathways, and thus can provide new potential treatment avenues for CF.

Acknowledgments—We thank SIDNET facility (SPARC BioCentre, The Hospital for Sick Children) for technical support, Dr. P. Karp from the University of Iowa Cell Culture Facility for ΔF508/ΔF508-CFTR HBE cells, and the Hubrecht Institute of Technology (HUB), Utrecht, for help with setting up the intestinal organoid cultures.

* This work was supported by the Canadian CF Foundation/Cystic Fibrosis Canada (CCFF/CFC), the Canadian Institute of Health Research (CIHR) and the Canadian Foundation for Innovation (CFI) (to DR). DR is a recipient of a CRC chair (Tier I) from the CFI.

** Both authors contributed equally to this work.

S This article contains supplemental Figs. S1 to S4 and Tables S1 to S4.

To whom correspondence should be addressed: The Hospital for Sick Children, PGCRL, 19-9715, 686 Bay St., Toronto, Ontario, Canada, M5G 0A4. Tel: (416) 813-5098; Email: drotin@sickkids.ca.

REFERENCES

1. Stutts, M. J., Canessa, C. M., Olsen, J. C., Hanrick, M., Cohn, J. A., Rossi, B. C., and Boucher, R. C. (1999) CFTR as a cAMP-dependent regulator of sodium channels. Science 269, 847–850

2. Boucher, R. C. (2007) Airway surface dehydration in cystic fibrosis: pathogenesis and therapy. Annu. Rev. Med. 58, 157–170

3. Davies, J. C., Atlon, E. W., and Bush, A. (2007) Cystic fibrosis. BMJ 335, 1255–1259

4. Riordan, J. R. (2008) CFTR function and prospects for therapy. Annu. Rev. Biochem. 77, 701–729

5. Quinton, P. M. (2010) Role of epithelial HCO3 transport in mucin secretion: lessons from cystic fibrosis. Am. J. Physiol. Cell Physiol 299, C1222–1233

6. Li, C., Ramjesingh, M., Wang, W., Garami, E., Hewryk, M., Lee, D., Rommens, J. M., Galley, K., and Bear, C. E. (1996) ATPase activity of the cystic fibrosis transmembrane conductance regulator. J. Biol. Chem. 271, 28463–28468

7. Dahan, D., Evagelidis, A., Hanranah, J. W., Hinks, D. A., Jia, Y., Luo, J., and Zhu, T. (2001) Regulation of the CFTR channel by phosphorylation. Pflugers Arch. 1, S92–96

8. Riordan, J. R., Rommens, J. M., Kerem, B-S., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenksi, J., Lok, S., Plavsic, N., Chou, J. L., Drumm, M. L., Iannuzzi, M. C., Collins, F. S., Lap-Tsu, C. (1989) Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 245, 1066–1073

9. Kerem, B., Rommens, J. M., Buchanan, J. A., Markiewicz, D., Cox, T. K., Chakravarti, A., Buchwald, M., and Tsu, L. C. (1989) Identification of the cystic fibrosis gene: genetic analysis. Science 245, 1073–1080

10. Cheng, S. H., Gregory, R. J., Marshall, J., Paul, S., Souza, D. W., White, G. A., O’Riordan, C. R., and Smith, A. E. (1990) Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. Cell 63, 827–834

11. Denning, G. M., Anderson, M. P., Amara, J. F., Marshall, J., Smith, A. E., and Welsh, M. J. (1992) Processing of mutant cystic fibrosis transmembrane conductance regulator is temperature-sensitive. Nature 358, 761–764

12. Kopito, R. R. (1999) Biosynthesis and degradation of CFTR. Physiol. Rev. 79, 5167–173

13. Serohijos, A. W., Hegedus, T., Aleksandrov, A. A., He, L., Cui, D., Dokholyan, N. V., and Riordan, J. R. (2008) Phenylalanine-508 mediates a cytoplasmic-membrane domain contact in the CFTR 3D structure crucial to assembly and channel function. Proc. Natl. Acad. Sci. U.S.A. 105, 3256–3261

14. Du, K., Sharma, M., and Lukacs, G. L. (2005) The DeltaF508 cystic fibrosis mutation impairs domain-domain interactions and arrests post-translation folding of CFTR. Nat. Struct. Mol. Biol. 12, 17–25

15. Rabeh, W. M., Bossard, F., Xu, H., Okiyoneda, T., Bagdany, M., Mulvihill, C. M., Du, K., di Bernardo, S., Liu, Y., Konermann, L., Roldan, A., and Lukacs, G. L. (2012) Correction of both NBD1 energetics and domain interface is required to restore DeltaF508 CFTR folding and function. Cell 148, 150–163

16. Sato, S., Ward, O. L., Krouse, M. E., Wine, J. J., and Kopito, R. R. (1996) Glycerol reverses the misfolding phenotype of the most common cystic fibrosis mutation. J. Biol. Chem. 271, 635–638

17. Galletta, L. J., Haggie, P. M., and Verkman, A. S. (2001) Green fluorescent protein-based halide indicators with improved chloride and iodide affinities. FEBS Lett. 499, 220–224

18. Ma, T., Thiragaraj, J. R., Yang, H., Sonawane, N. D., Folli, C., Galletta, L. J., and Verkman, A. S. (2002) Thiazolidine-4-carboxylic acid inhibitor identified by high-throughput screening blocks cholera toxin-induced intestinal fluid secretion. J. Clin. Invest. 110, 1651–1658

19. Ma, T., Vetrivel, L., Yang, H., Pedemonte, N., Zegarra-Moran, O., Galletta, L. J., and Verkman, A. S. (2002) High-affinity activators of cystic fibrosis transmembrane conductance regulator (CFTR) chloride conductance identified by high-throughput screening. J. Biol. Chem. 277, 37235–37241

20. Yang, H., Shelat, A. A., Guy, R. K., Gopinath, V. S., Ma, T., Du, K., Lukacs, G. L., Taddei, A., Folli, C., Pedemonte, N., Galletta, L. J., and Verkman, A. S. (2003) Nanomolar affinity small molecule correctors of defective Delta F508-CFTR chloride channel gating. J. Biol. Chem. 278, 35079–35085
32. Sato, T., Stange, D. E., Ferrante, M., Vries, R. G., Van Es, J. H., Van den Droebner, K., and Sandner, P. (2013) Modification of the salivary secretion of human colon, adenoma, adenocarcinoma, and Barrett’s epithelium. J. Cyst. Fibros. 12, 273–280.

33. Dekkers, J. F., Wiegerinck, C. L., de Jonge, H. R., Bronsveld, I., Janssens, J. C., Bijlenga, H., Maling, L. M., Miller, M., Neugebauer, T., Olson, E., Panchenko, V., Rader, J., Singh, A., Stack, J. H., Tung, R., Grootenhuis, P. D., and Negulescu, P. (2006) Rescue of DeltaF508-CFTR trafficking and gating in human cystic fibrosis airway primary cultures by small molecules. Am. J. Physiol. Lung Cell Mol. Physiol. 290, L1117–1130.

34. Clancy, J. P., Rowe, S. M., Accurso, F. J., Atkisson, M. L., Anin, R. S., Astwood, M. A., Ballmann, M. B., Boyle, M. P., Bronsveld, I., Campbell, P. W., Deboeck, K., Donaldson, S. H., Dorkin, H. L., Dunitz, J. M., Durie, P. R., Jain, M., Leonard, A., McCoy, K. S., Moss, R. B., Pilewski, J. M., Rosenbluth, D. B., Rubenstein, R. C., Schechter, M. S., Botfield, M., Ordonez, C. L., Spencer-Green, G. T., Vennilet, L., Wisseh, S., Yen, K., and Konstan, M. W. (2012) Results of a phase IIa study of VX-809, an investigational CFTR corrector compound, in subjects with cystic fibrosis airway epithelium. J. Cyst. Fibros. 11, 1439–1452.

35. Meacham, G. C., Lu, Z., King, S., Sorscher, E., Tousson, A., and Cystic Fibrosis Foundation (2011) Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett’s epithelium. J. Cyst. Fibros. 10, 630–637.

36. Norez, C., Bilan, F., Kitizis, A., Mettey, Y., and Bégouën, A. (2002) The role of fibroblast growth factor receptors in carcinogenesis. Mol. Pharmacol. 63, 645–657.

37. Turner, N., and Grose, R. (2010) Fibroblast growth factor signalling: from development to cancer. Nat. Rev. Mol. Cell Biol. 11, 55–67.

38. Ovsenek, N., Chomyn, A., and Housman, D. (1998) Transcriptional activation by heat shock factor 1: A general role for the heat shock factor 1 family in cellular stress responses. Science 280, 939–945.

39. Nakai, A., Suzuki, M., and Tanabe, M. (2000) Arrest of spermatogenesis in mice expressing an active heat shock transcription factor 1. EMBO J. 19, 1545–1554.

40. Droebner, K., and Sandner, P. (2013) Modification of the salivary secretion assay in F508del mice – The murine equivalent of the human sweat test. J. Cyst. Fibros. 12, 630–637.

41. Sato, T., and Clevers, H. (2013) Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. Science 340, 1190–1194.

42. Choln, D. M., Quinney, N. L., Fulcher, M. L., Esther, C. R., Jr., Das, J., Dokholyan, N. V., Randell, S. H., Boucher, R. C., and Gentzsch, M. (2014) Potentiator ivacaftor abrogates pharmacological correction of DeltaF508 CFTR in cystic fibrosis. Sci. Transl. Med. 6, 246ra296.

43. Veit, G., Avramescu, R. G., Perdomo, D., Phuan, P. W., Bagdady, M., Apaja, P. M., Borot, F., Szollosi, D., Wu, Y. S., Finkbeiner, W. E., Hegedus, T., Verkman, A. S., and Lukacs, G. L. (2014) Some gating potentiators, including VX-770, diminish DeltaF508-CFTR functional expression. Sci. Transl. Med. 6, 246ra297.

44. Johnson, L. G., Olsen, J. C., Sarkadi, B., Moore, K. L., Swanstrom, R., and Boucher, R. C. (1992) Efficiency of gene transfer for restoration of normal airway epithelial function in cystic fibrosis. Nat. Genet. 2, 21–25.

45. Zhang, L., Button, B., Gabriel, S. E., Burket, S., Yan, Y., Skiadopoulos, H. D., Patte, Y. L., Van, C. M., Thomas, D. Y., and Konstan, M. W. (2012) Results of a phase IIa study of VX-809, an investigational CFTR corrector compound, in subjects with cystic fibrosis airway epithelium. PLoS Biol 7, e1000155.

46. Javerzat, S., Auguste, P., and Bilkafalvi, A. (2002) The role of fibroblast growth factors in vascular development. Trends Mol. Med. 8, 483–489.

47. Turner, N., and Grose, R. (2010) Fibroblast growth factor signalling: from development to cancer. Nat. Rev. Mol. Cell Biol. 11, 55–67.

48. Haugsten, E. M., Wiedlocha, A., Olesne, S., and Wesche, J. (2010) Roles of fibroblast growth factor receptors in carcinogenesis. Mol. Cancer Res. 8, 1439–1452.

49. Oklonyeda, T., Barriere, H., Bagdady, M., Rabe, W. M., Du, K., Hohfeld, J., Young, J. C., and Lukacs, G. L. (2010) Peripheral protein quality control removes unfolded CFTR from the plasma membrane. Science 329, 999–1003.

50. Meacham, G. C., Patterson, C., Zhang, W., Younger, J. M., and Cyster, D. J. (2001) The Hsc70 chaperone CHIP targets immature CFTR for proteasomal degradation. Nat. Cell Biol. 3, 100–105.

51. Younger, J. M., Chen, L., Ren, H. Y., Rossor, M. F., Turnbull, E. L., Fan, C. Y., Patterson, C., and Cystic Fibrosis Foundation (2006) Structural quality-control checkpoints triad misfolded cystic fibrosis transmembrane conductance regulator. Cell 126, 571–582.

52. Wang, X., Venable, J., LaPointe, P., Hutt, D. M., Koulou, A. V., Copping, J., Gurkan, C., Keliner, W., Matteson, J., Plutner, H., Riordan, J. R., Kelly, J. W., Yates, J. R., 3rd, and Balch, W. E. (2006) Hsps90 chaperones Aha1 downregulation rescues misfolding of CFTR in cystic fibrosis. Cell 125, 803–815.

53. Norez, C., Bilan, F., Kitizis, A., Mettey, Y., and Becq, F. (2008) Proteasome-dependent pharmacological rescue of cystic fibrosis transmembrane conductance regulator revealed by mutation of glycine 622. J. Pharamacol. Exp. Ther. 325, 89–99.

54. Fulmer, W., and Cuthbert, A. W. (2000) Post-translational disruption of the delta F508 cystic fibrosis transmembrane conductance regulator (CFTR)-molecular chaperone complex with geldanamycin stabilizes delta F508 CFTR in the rabbit reticulocyte lysate. J. Biol. Chem. 275, 29147–29152.

55. Banerjee Mustafi, S., Chakraborty, P. K., and Raha, S. (2010) Modulation of Akt and ERK1/2 pathways by resveratrol in chronic myelogenous leuke- mia (CML) cells results in the downregulation of Hsp70. PLoS One 5, e8719.
68. Kuhl, M., Sheldahl, L. C., Park, M., Miller, J. R., and Moon, R. T. (2000) The
66. Ikeda, A., Masaki, M., Kozutsumi, Y., Oka, S., and Kawasaki, T. (2001)
67. Masaki, M., Ikeda, A., Shiraki, E., Oka, S., and Kawasaki, T. (2003) Mixed
64. Craig, E. A., Stevens, M. V., Vaillancourt, R. R., and Camenisch, T. D. (2008)
69. Ishitani, T., Kishida, S., Hyodo-Miura, J., Ueno, N., Yasuda, J., Waterman,
71. Jakob, U., Gaestel, M., Engel, K., and Buchner, J. (1993) Small heat shock
70. Walsh, D., Li, Z., Wu, Y., and Nagata, K. (1997) Heat shock and the role of
57. Murapa, P., Gandhapudi, S., Skaggs, H. S., Sarge, K. D., and Woodward,
56. Bao, X. Q., and Liu, G. T. (2001) Induction of HSP70 promotes
58. Rada, A., Escotte, S., Couetil, J. P., Cornillet, P., Gue
59. Saito, K., Dai, Y., and Zeiltin, P. L. (2001) Induction of HSP70 promotes
72. Kostenko, S., and Moens, U. (2009) Heat shock protein 27 phosphorylation:
73. Garrido, C., Paul, C., Seigneuric, R., and Kampinga, H. H. (2012) The small
74. Ahner, A., Gong, X., Schmidt, B. Z., Peters, K. W., Rabeh, W. M., Thibo-
dauea, P. H., Lukacs, G. L., and Frizzell, R. A. (2013) Small heat shock
52. Craig, E. A., Stevens, M. V., Vaillancourt, R. R., and Camenisch, T. D. (2008)
51. Craig, E. A., Stevens, M. V., Vaillancourt, R. R., and Camenisch, T. D. (2008)
50. Craig, E. A., Stevens, M. V., Vaillancourt, R. R., and Camenisch, T. D. (2008)
49. Craig, E. A., Stevens, M. V., Vaillancourt, R. R., and Camenisch, T. D. (2008)
48. Craig, E. A., Stevens, M. V., Vaillancourt, R. R., and Camenisch, T. D. (2008)

Molecular & Cellular Proteomics 14.6

1583