Esc peptides for novel therapeutics against *Pseudomonas aeruginosa* pulmonary infections: beyond antimicrobial activity

Maria Rosa Loffredo[1], Loretta Ferrera[2], Floriana Cappiello[1], Bruno Casciaro[1], Giacomo Cappella[1], Ivana D'Angelo[3], Y. Peter Di[4] , Maria Luisa Mangoni[1]

[1]Department of Biochemical Sciences, Sapienza University of Rome; [2]U.O.C. Genetica Medica, Istituto Di Ricovero e Cura a Carattere Scientifico, Istituto Giannina Gaslini, Genova; [3]Department of Biotechnology, DiSTABiF, University of Campania Luigi Vanvitelli, Caserta, Italy;

[4]Department of Environmental and Occupational Health, University of Pittsburgh, USA

Email: marialuisa.mangoni@uniroma1.it

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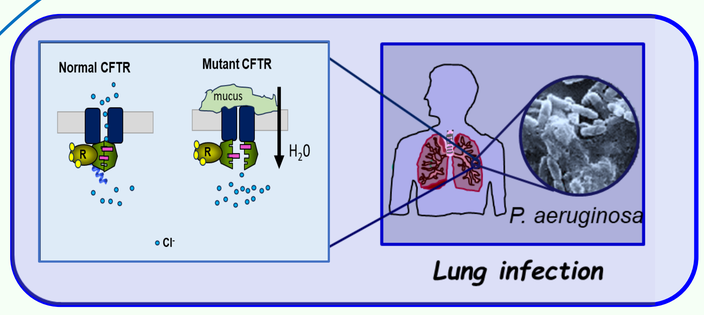
BACKGROUND & RESULTS



- **Cystic fibrosis (CF) is a genetic disorder due to mutations in the gene encoding the CFTR channel at the apical membrane of epithelia, including those at the airways [1,2].**

- **The most prevalent mutation is the loss of phenylalanine 508 (F508del CFTR) which severely impairs CFTR trafficking to the cell surface and alters the mechanisms of channel opening. Despite the clinical efficacy of present CFTR modulators in restoring the activity of defective CFTR, some patients remain untreatable or continue to experience chronic pulmonary infections, presenting considerable challenges.**

- **In the last years, we identified two antimicrobial peptides (AMPs), Esc peptides [3-5] (scheme 1), that (i) rapidly kill *P. aeruginosa* and eradicate its biofilm with a membrane-perturbing activity that prevents bacteria from developing resistance; (ii) accelerate recovery of damaged bronchial epithelium; (iii) reduce lung bacterial burden in C57BL/6 mice upon intra-tracheal instillation at a very low dosage (0.1 mg/kg) (Figure 1).**

Note that complete activation of CFTR with gating mutations can be achieved upon addition of forskolin (FSK), which promotes the phosphorylation of the channel, and genistein (GEN) to prolong the open channel probability [6,7].

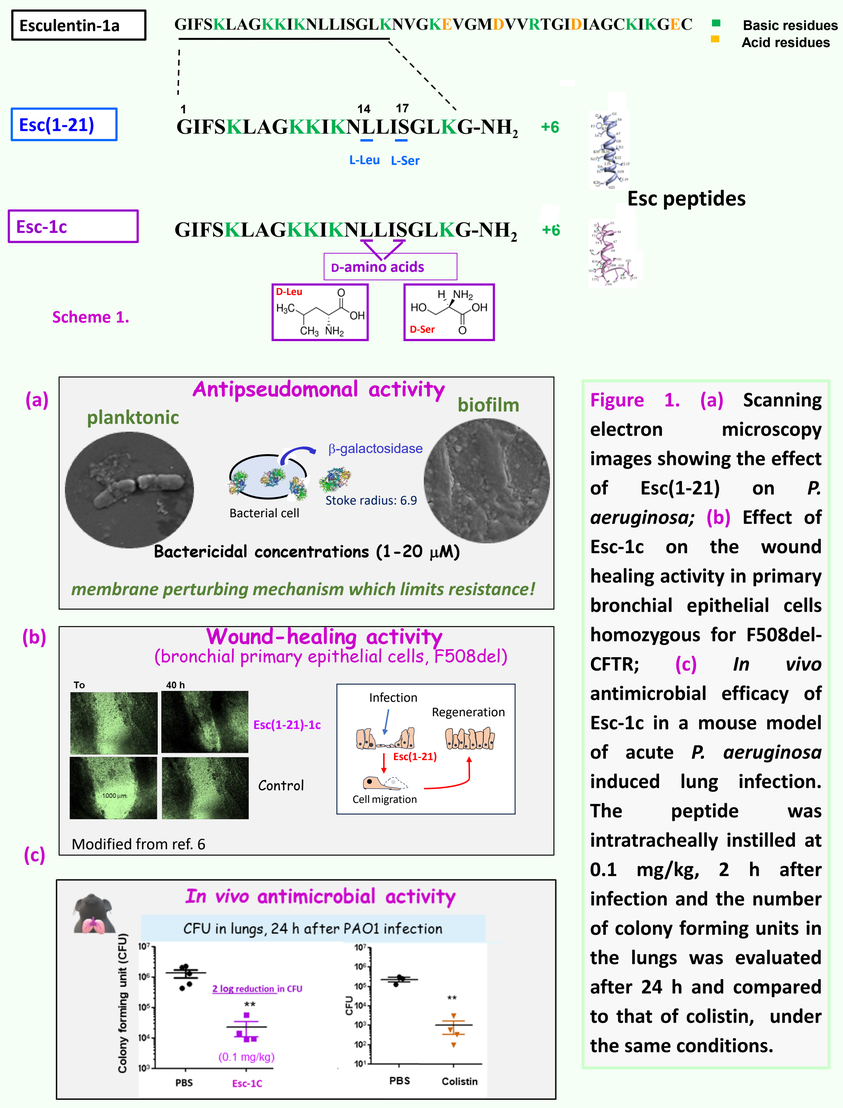


Figure 1. (a) Scanning electron microscopy images showing the effect of Esc(1-21) on *P. aeruginosa*; (b) Effect of Esc-1c on the wound healing activity in primary bronchial epithelial cells homozygous for F508del-CFTR; (c) *In vivo* antimicrobial efficacy of Esc-1c in a mouse model of acute *P. aeruginosa* induced lung infection. The peptide was intratracheally instilled at 0.1 mg/kg, 2 h after infection and the number of colony forming units in the lungs was evaluated after 24 h and compared to that of colistin, under the same conditions.

Recently, the ability of Esc peptides to increase the F508del CFTR-mediated transepithelial conductance (TEEC) was studied in combination with FSK. This effect was higher for Esc-1C at 10 μM and comparable to that of GEN when used at the same concentration (Figure 2). We also found that this effect is highly dependent on the primary structure of Esc peptides and the presence of Ser-17 (Figure 3).

The CFTR potentiating activity of Esc peptides was well preserved in primary airway epithelial cells from homozygous F508del patients (Figure 4).

To verify whether the effect of Esc peptides on the ion currents was controlled by CFTR, patch clamp experiments (*inside-out configuration*) were carried out on Fisher Rat Thyroid cells (FRT) expressing F508del CFTR. The increased peptide-induced ion current is due to activation of CFTR and likely mediated by direct interaction of the peptides with CFTR (Figure 5).

To achieve the lungs, it is fundamental the development of pharmaceutical formulations able to assist the peptide diffusion through extracellular barriers and to provide a gradual peptide release at the infectious site.

Peptide-loaded PLGA-PVA nanoparticles (NPs) were able to improve the diffusion of the encaspulated peptide through the bronchial mucus and simulated biofilm (Figure 6).

Note also that peptide-loaded NPs were found to be more efficient in reducing the number of *P. aeruginosa* in a mouse model of acute lung infection compared to the results found when the peptides were i.t. instilled in their soluble free form (Figure 7).

CFTR-DEPENDENT TRANSEPITHELIAL CONDUCTANCE (ΔG) BY ESC PEPTIDES

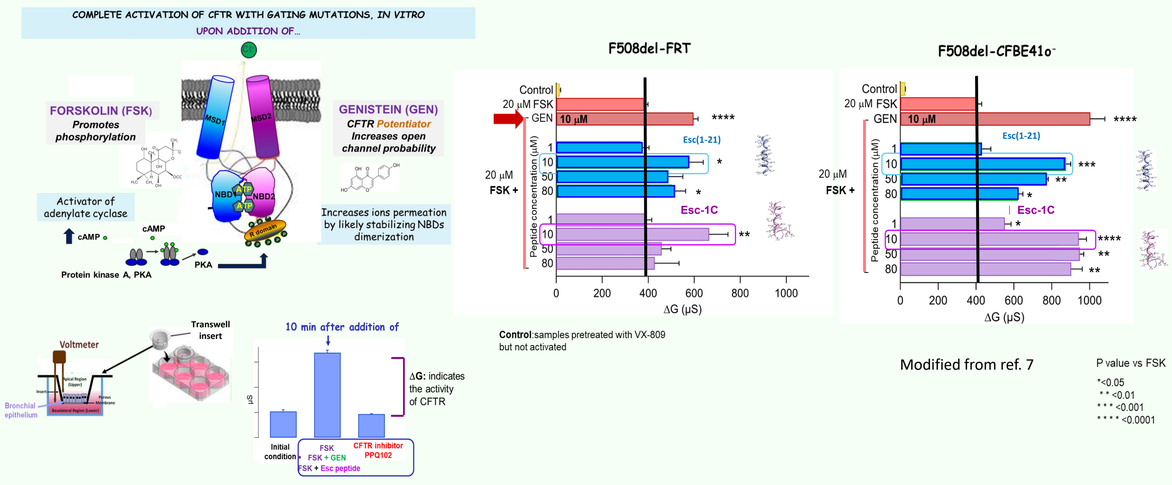


Figure 2. Fisher Rat Thyroid (FRT) cells or bronchial epithelial cells (CFBE41o$^-$) expressing F508del-CFTR were pre-incubated with the corrector VX-809 (to deliver the mutated protein to the membrane) for 24h and then with Esc peptides for 10 min at different concentrations, in the presence of FSK. Then, CFTR channel activity was fully inhibited by the CFTR specific inhibitor PPQ-102 (30 μM) and CFTR-mediated transepithelial electrical conductance was calculated.

CFTR-DEPENDENT TRANSEPITHELIAL CONDUCTANCE (ΔG) BY ANALOGS OF ESC PEPTIDES

Sequence
1 10 20 Gly-Ile-Phe-Ser-Lys-Leu-Ala-Gly-Lys-Lys-Ile-Lys-Asn-D*Leu*-Leu-Ile-Ala-Gly-Leu-Lys-Gly-NH_2
1 10 20 Gly-Ile-Phe-Ser-Lys-Leu-Ala-Gly-Lys-Lys-Ile-Lys-Asn-D*Leu*-Leu-Ile-D*Ala*-Gly-Leu-Lys-Gly- NH_2
1 10 20 Gly-Ile-Phe-Ser-Lys-Leu-Ala-Gly-Lys-Lys-Ile-Lys-Asn-D*Leu*-Leu-Ile-Gly-Gly-Leu-Lys-Gly-NH_2
1 10 20 Gly-Ile-Phe-Ser-Lys-Leu-Ala-Gly-Lys-Lys-Ile-Lys-Asn-D*Leu*-Leu-Ile-D*Thr*-Gly-Leu-Lys-Gly- NH_2
1 10 20 Gly-Ile-Phe-Ser-Lys-Leu-Ala-Gly-Lys-Lys-Ile-Lys-Asn-D*Leu*-Leu-Ile-Thr-Gly-Leu-Lys-Gly- NH
1 10 20 Gly-Ile-Phe-Ser-Lys-Leu-Ala-Gly-Lys-Lys-Ile-Lys-Asn-D*Leu*-Leu-Ile-D*Phe*-Gly-Leu-Lys-Gly-NH_2
1 10 20 Gly-Ile-Phe-Ser-Lys-Leu-Ala-Gly-Lys-Lys-Ile-Lys-Asn-D*Leu*-Leu-Ile-Phe-Gly-Leu-Lys-Gly-NH_2
1 10 20 Gly-Ile-Phe-Ser-Lys-Leu-Ala-Gly-Lys-Lys-Ile-Lys-Asn-D*Leu*-Leu-Ile-D*phosphoSer*-Gly-Leu-Lys-Gly-NH_2

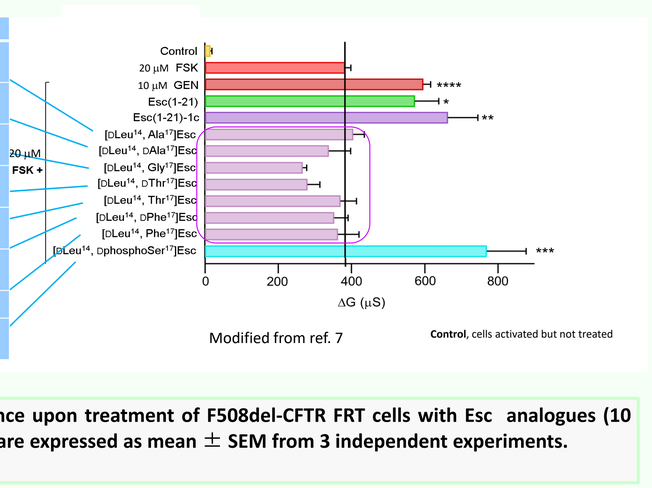


Figure 3. CFTR-mediated transepithelial conductance upon treatment of F508del-CFTR FRT cells with Esc analogues (10 μM) for 10 min in the presence of 20 μM FSK. Data are expressed as mean ± SEM from 3 independent experiments. *, $p < 0.01$; ** $p < 0.001$; ** p<0.0001, vs FSK.

CFTR-DEPENDENT TRANSEPITHELIAL CONDUCTANCE (ΔG) IN PRIMARY BRONCHIAL EPITHELIA

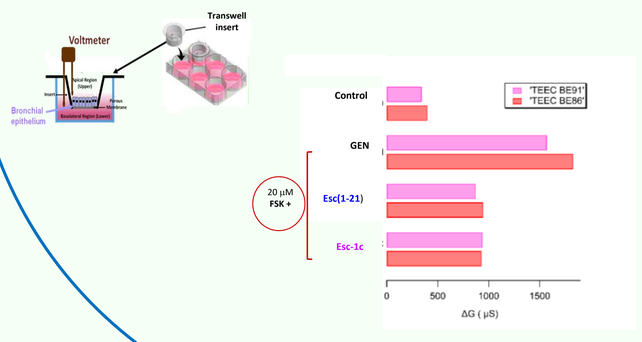


Figure 4. Effect of peptides on CFTR-mediated TEEC in primary airway epithelial cells obtained from a CF patient homozygous for the mutation F508del. TEEC was evaluated as the difference between the conductance measured after 10 min addition of 20 μM FSK alone or after addition of the combination FSK + GEN or peptides to Vx-809 pre-incubated epithelia, and the conductance measured after CFTR inhibition (delta conductance, ΔG) upon addition of PPQ102, as reported in Fig. 2. Epithelia pre-incubated with VX-809 but not activated (control) were included for comparison.

EFFECT OF PEPTIDES ON IONS CURRENT BY PATCH CLAMP EXPERIMENTS (inside-out configuration)

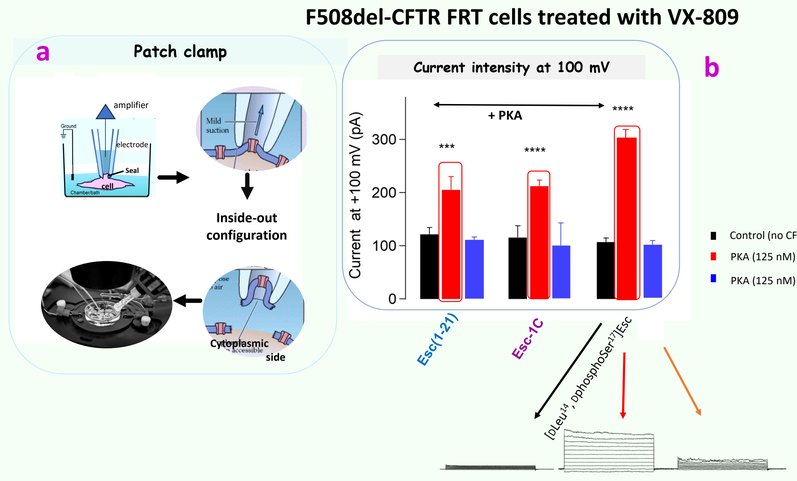


Figure 5.
a. Current intensity at +100 mV for indicated treatment. ****, $p < 0.0001$ vs control. Superimposed currents elicited at different membrane potential are also indicated for the phosphorylated peptide. b. Current ratio at +100 mV upon addition of protein kinase A (pKA) and ATP.

NPs DIFFUSION THROUGH ARTIFICIAL BIOLOGICAL BARRIERS & KINETICS OF PEPTIDE RELEASE

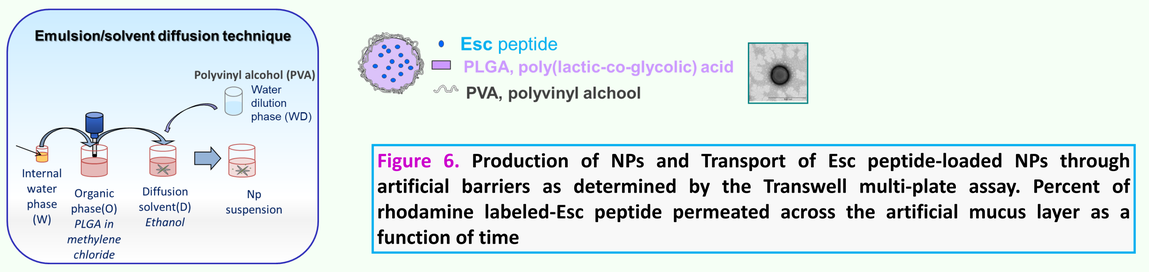


Figure 6. Production of NPs and Transport of Esc peptide-loaded NPs through artificial barriers as determined by the Transwell multi-plate assay. Percent of rhodamine labeled-Esc peptide permeated across the artificial mucus layer as a function of time

EFFICACY IN MURINE MODELS OF ACUTE LUNG INFECTION AT 24h AFTER BACTERIAL CHALLENGE

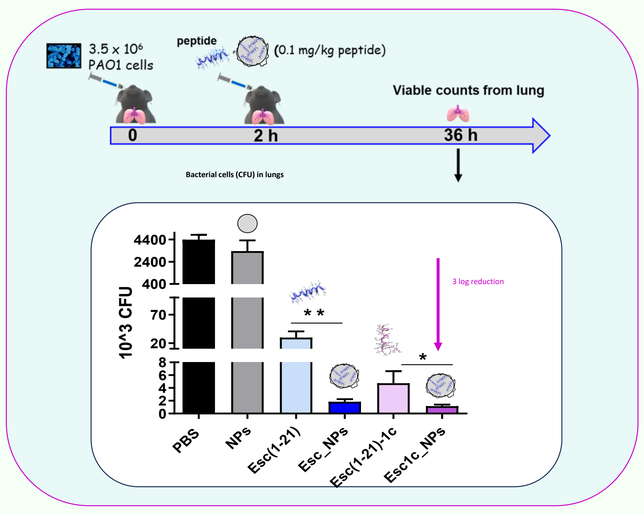


Figure 7. Comparison among bare NPs, Esc peptides and peptides-loaded NPs on the number of viable *P. aeruginosa* cells (CFU) in mouse lungs at 36 h after their administration. Results are the mean ± SEM from three independent experiments. The t-test was used to compare the means of peptide-treated infected mice versus animals treated with the corresponding peptide-loaded NPs. The levels of statistical significance are *p* values of< 0.05 (*) and < 0.01 (**).

CONCLUSIONS & PERSPECTIVES

These studies should allow the development of new peptide-based therapeutic agents able to:

- ***Defeat P. aeruginosa lung infection***
- ***Heal damaged bronchial epithelium***
- ***Restor the function of mutated CFTR***

Advantageous properties for treatment of Lung pathology in CF !

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