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Editorial Board

169

Targeting microRNAs in cystic fibrosis (CF)

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Cystic fibrosis (CF) is a lethal autosomal recessive genetic disease caused by a variety of mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Since the demonstration that microRNAs are deeply involved in CF, increasing attention has been dedicated to the possible alteration of CFTR gene expression by targeting those miRNAs involved in the downregulation of CFTR and associated proteins. In this case, peptide nucleic acids (PNAs) appear to be of great interest, since they are capable of sequence-specific and efficient hybridization with complementary DNA and RNA. Our group has demonstrated that the PNA-mediated inhibition of miR-145-5p (1) and miR-101-3p (which downregulate CFTR) leads to an increase in CFTR expression in Calu-3 cells. In addition, to the direct interaction with CFTR, miRNAs may regulate CFTR by binding to the 3'UTR of mRNAs coding CFTR regulators, such as NHERF1, NHERF2 and ezrin. Notably, the PNA-mediated targeting of miR-335-5p, one of the miRNAs involved in NHERF1 regulation, was found to be associated with the specific inhibition of miR-335-5p, an increase in NHERF1 and an increase in CFTR expression. NGS was performed to verify whether PNA-mediated effects may be accompanied by the co-inhibition of other miRNAs. The results obtained sustain the concept that specific miRNA inhibition (in our case inhibition of miR-145-5p, miR-101-3p and miR-335-5p) may be accompanied by the co-inhibition of other miRNAs (for instance miR-155-5p in the case of PNA-mediated inhibition of miR-145-5p) involved in cystic fibrosis (2). In addition to the PNA-mediated targeting of CFTR-inhibiting miRNAs (anti-miRNA therapeutic approaches), miRNA-replacement therapy may also be considered. This may be a key strategy for inhibiting *Pseudomonas aeruginosa*-dependent inflammatory responses, with relevant clinical implications. In this respect, miR-93-5p was demonstrated to be downregulated during the *P. aeruginosa* infection of CF cells (3). Accordingly, the transfection of CF cells with pre-miR-93-5p was found to be associated with anti-inflammatory effects, including a decrease in IL-8 mRNA content and IL-8 protein release.

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170

A peptide nucleic acid targeting the *acpP* gene of *Pseudomonas aeruginosa* inhibits bacterial induced biological alterations in cystic fibrosis cells

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One of the major clinical features of Cystic Fibrosis (CF) is the chronic infection generally sustained by the Gram-negative bacterium *Pseudomonas aeruginosa (P.aeruginosa)*[(1)]. An excessive lung inflammation, with a huge infiltrate of neutrophils in the bronchial lumen, CF is associated with *P.aeruginosa* infection and occurs mainly due to the release of the chemokine interleukin IL-8[(2)]. The identification of new effective antibacterial drugs, able to reverse, at least partially, the *P.aeruginosa*-induced alteration of biological effects on CF cells, is considered a promising therapeutic strategy to prevent progressive lung tissue deterioration. Recently, antisense antibacterial Peptide Nucleic Acids (PNAs) targeting the mRNA translation initiation region of *ftsZ* (an essential bacterial gene involved in cell division) or *acpP* (an essential bacterial gene involved in fatty acid synthesis) of *P.aeruginosa* were discovered, and showed promising bactericidal activity[(3)]. The aim of the present study was to determine the effects of one of these anti-*acpP* PNA peptide conjugates (PNA-3969) on *P.aeruginosa* (PAO-1 strain) induction of the biological effects on CF cells. The first conclusion of the present study is that this PNA protects NuLi-1 cells, CF IB3-1 and CuFi-1 cells from the bacterial induced upregulation of the pro-inflammatory cytokine IL-8. Moreover, the anti-PAO-1 PNA-3969 protects IB3-1 cells from bacterial-induced cytotoxicity and pro-apoptotic effects, with an efficiency approaching that of the antibacterial agent gentamycin. Of interest, cooperative effects were obtained when PNA-3969 and gentamycin were used in combination at sub-optimal concentrations. In conclusion, this type of antisense antibiotics should be considered of great interest for further discovery of novel and specific antibiotics against pulmonary infections in cystic fibrosis patients.

Acknowledgements: This work was supported by grants from the Italian Cystic Fibrosis Research Foundation (grant nos. 15/2004 and 17/2010 to R.G.).

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171

New synthetic isoxazole derivatives as potent inducer of fetal hemoglobin (HbF) in β-thalassemia

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Several examples linking anti-proliferative effects to erythroid differentiation using many cellular model systems (for instance K562 cells) do exist. Moreover, in most cases, inducers of K562 erythroid differentiation are capable of stimulating fetal hemoglobin (HbF) production in erythroid precursor cells (ErPCs) isolated from β-thalassemia patients. In this study, new anti-proliferative 4,5,6,7-tetrahydro-isoxazolo-[4,5-c]-pyridines and 3,4-isoxazolediamide derivatives[(1)] were screened for induction of erythroid differentiation in K562 cells and stimulation of HbF induction in ErPCs. The original structures of geldanamycin and radicicol, considered natural Hsp inhibitors, were modified because both of them exhibit several drawbacks, including poor solubility, significant hepatotoxicity and intrinsic chemical instability or deprivation of *in vivo* activity. These potential HbF inducers were tested first on the K562 cell line and second on erythroid precursors derived from patients affected by β-thalassemia and SCD (Sickle Cell Disease) in order to select novel bioactive analogues. Increase of HbF was determined by HPLC and γ-globin mRNA expression was analyzed by RT-qPCR. Overall, the data obtained indicate that: (a) Isoxazol derivatives increase HbF in cultures from β-thalassemia and SCD patients with different basal HbF levels; (b) Isoxazol derivatives increase the overall Hb content/cell; (c) Isoxazol derivatives selectively induce γ-globin mRNA accumulation, with only a minor effect on β-globin and no effect on α-globin mRNAs; (d) there is a strong correlation between the increase by Isoxazol derivatives of HbF and the increase in γ-globin mRNA content; (e) in several cases these compounds were found more active than hydroxyurea, a well-known HbF inducers in β-thalassemia and SCD.

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172

A PNA-based masking strategy for CFTR upregulation by targeting miR-145-5p binding sites of CFTR mRNA

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MicroRNA miR-145-5p is involved in the post-transcriptional regulation of the Cystic Fibrosis Transmembrane conductance Regulator (*CFTR*) gene, deeply altered in Cystic Fibrosis (CF). We have recently reported the effects of a Peptide Nucleic Acid (PNA) targeting miR-145-5p (1,2). An octaarginine-anti-miR145 PNA conjugate was delivered to Calu-3 cells, exerting sequence-dependent inhibition of miR-145-5p, associated with enhancement of the expression of the miR-145 regulated *CFTR* gene, analyzed at mRNA (RT-qPCR, Reverse Transcription quantitative Polymerase Chain Reaction) and CFTR protein (western blotting) level. In addition to this anti-miRNA strategy, PNAs can be employed as useful tools to perform efficient 'masking' of miRNA binding sites, thus preventing molecular interactions between miRNAs and target 3'UTR binding sites. In this context, we designed, synthesized and tested a PNA 100% complementary to the CFTR mRNA 3'UTR, (N-term, 5')-R8-CCA GTT ATC ATT ATC TAA-Gly-NH2(C-term, 3') (R8-PNA-145mask). Calu-3 cells were treated with increasing concentrations of the R8-PNA-145mask (0.5-4 μM) for three days, and RNA and proteins were isolated from treated cells to analyze the *CFTR* gene expression. The results obtained demonstrated that the treatment of Calu-3 cells with the R8-PNA-145mask leads to an increase of *CFTR* expression. Therefore, direct inhibition of miR-145-5p with antisense PNAs and/or PNA-dependent prevention of miR-145-5p binding to the CFTR mRNA 3'UTR might be efficient strategies to enhance CFTR expression, a key action in therapeutic protocols for CF.

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