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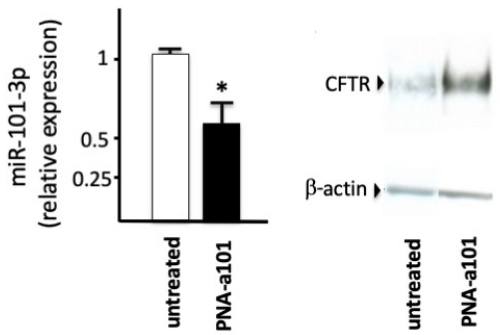
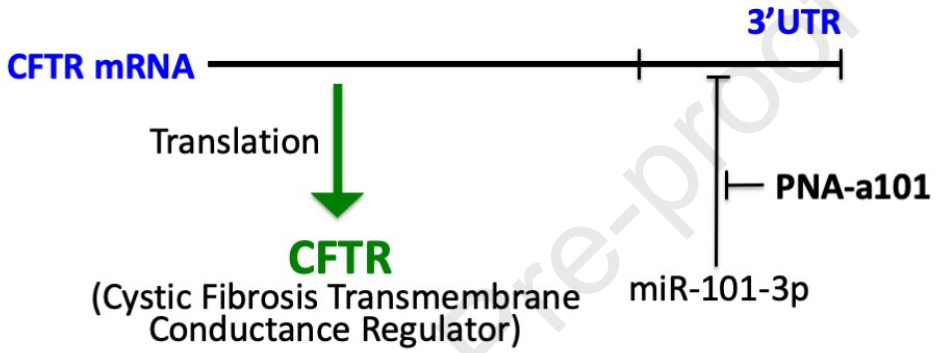


MicroRNA miR-101-3p targets the 3'-UTR of the *CFTR* mRNA

A peptide nucleic acid (PNA) targeting miR-101-3p (PNA-a101) inhibits miR-101-3p leading to an increase of *CFTR* gene expression



Treatment of human airway epithelial Calu-3 cells with a Peptide-Nucleic Acid (PNA) targeting the microRNA miR-101-3p is associated with increased expression of the Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene

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Key words: Peptide nucleic acids; cystic fibrosis; microRNAs; miR-101-3p; miRNA targeting; delivery; CFTR.

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ABSTRACT

Since the identification of microRNAs (miRNAs) involved in the regulation of Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene, miRNAs known to down-regulate the expression of the CFTR and associated proteins have been investigated as potential therapeutic targets. Here we show that miR-101-3p, targeting the 3'-UTR sequence of the *CFTR* mRNA, can be selectively inhibited by a peptide nucleic acid (PNA) carrying a full complementary sequence. With respect to clinical relevance of microRNA targeting, it is expected that reduction of concentration of miRNAs (the anti-miRNA approach) could be associated with increasing amounts of target mRNAs. Consistently to this hypothesis, we report that PNA-mediated inhibition of miR-101-3p was accompanied by CFTR up-regulation. Next Generation Sequencing (NGS) was performed in order to verify the effects of the anti-miR-101-3p PNA on the Calu-3 miRNome. Upon inhibition of miR-101-3p we observed a fold change (FC) expression <2 of the majority of miRNAs (403/479, 84.13%), whereas we identified a list of dysregulated miRNAs, suggesting that specific miRNA inhibition (in our case miR-101-3p) might be accompanied by alteration of expression of other miRNAs, some of them known to be involved in Cystic Fibrosis (CF), such as miR-155-5p and miR-125b-5p.

1. Introduction

Among the several regulatory networks controlling gene expression, those regulated by non-coding RNAs appear to be of great interest and involve possible novel molecular targets for therapeutic interventions. Among non-coding RNAs, microRNAs (miRNAs) (exhibiting a length comprised between 19 to 25 nucleotides) are key players. They interact with mRNAs by recognizing the 3'-UTR region at the level of the RNA-induced silencing complex (RISC), causing translational repression or mRNA degradation [1,2]. Depending on these mechanisms of action, miRNAs regulate complex biological functions, such as differentiation, cell cycle and apoptosis [3,4].

Cystic Fibrosis (CF) is the most common life-threatening rare disease among Caucasians, occurring in approximately one in 3,000–4,000 live birth and caused by genetic defects of the CF Transmembrane Conductance Regulator (*CFTR*) gene [5].

In the field of translational research on CF, miRNA-based regulation of the expression of the *CFTR* gene and of CFTR-regulators has been recently demonstrated [6-15] (Table I). In this respect, miR-101-3p is one of the validated microRNAs controlling *CFTR* gene expression [7,11-13]. For instance, Hassan et al. reported that miR-101-3p regulates the expression of the CFTR in the lung [7]. When a miR-101-3p precursor was transfected to human airway epithelial cells, it directly targeted the 3'-UTR of the *CFTR* mRNA and suppressed the expression of the CFTR protein. Interestingly, miR-101-3p is also deeply involved in *CFTR* regulation in lung samples from patients with severe chronic obstructive pulmonary disease (COPD), where this microRNA is overexpressed when compared to control patients [7]. This leads to a suppression and/or down-regulation of CFTR. Interestingly, Megiorni et al. found a synergism between miR-101-3p and miR-494 in post-transcriptional regulation of the *CFTR* gene [13]. The effect on *CFTR* was supported by the finding that both miR-101 and miR-494 mimics significantly inhibited the expression of a reporter construct containing the 3'-UTR of

CFTR in luciferase assays. Moreover, a miR-101/miR-494 combination was able to markedly suppress CFTR activity, to an extent higher than that found in the case of treatments with single miRNAs [13]. According to this report, aberrant expression and function of miRNAs might explain the wide phenotypic variability observed among CF patients. This opens new avenues in the field of precision medicine in CF, i.e. the targeting of overexpressed miRNAs, providing that a careful analysis of their expression pattern is performed. This is particularly important in *CFTR* gene regulation, since *CFTR* mRNA is potential target of several microRNAs, including miR-433-3p, miR-509-3p, miR-335-5p, mir-132-3p, miR-101-3p, miR-144-3p, miR-494-3p, miR-223-3p, miR-145-5p (see Table I) [14,15]. Furthermore, microRNAs have been suggested to negatively regulate the expression of several genes coding proteins stabilizing CFTR on the apical membrane of bronchial epithelial cells [16-19]. For instance, the Na(+)/H(+) Exchange Regulatory Factors 1 (NHERF1) [16] and 2 (NHERF2) [17] interact with the cytoskeleton via ezrin [18,19], suggesting that they may help in anchoring CFTR to the cytoskeleton at the apical membrane. In addition to miRNAs binding to the 3'-UTR of *CFTR* mRNA, Table I shows examples of miRNAs regulating *NHERF1*, *NHERF2* and *ezrin* mRNAs [14,15]. Finally, in addition to miRNAs targeting CFTR and CFTR regulators, the expression of other miRNAs is altered in CF (see Table II for a partial list) [7-13, 20-33].

In the field of therapeutic molecules targeting microRNAs, Peptide Nucleic Acids (PNAs) have been described as DNA analogues exhibiting several properties of great interest in translational research [34-40]. Despite a radical structural change with respect to DNA and RNA, as they incorporate a backbone constituted of N-(2-aminoethyl)glycine units that replaces the natural ribose/phosphate polymer, PNAs are capable of an efficient sequence-specific hybridization with complementary DNA and RNA, generating Watson-Crick double helices [34-36]. In addition, they are able to participate in triple helix structures with double stranded DNA and to perform strand invasion [37]. Accordingly, PNAs have been extensively used for *in vitro* and *in vivo* pharmacologically-mediated

alteration of gene expression [35,38-40]. For instance, PNA and PNA-based analogues have been proposed as antisense molecules targeting mRNAs, artificial promoters, triple-helix forming molecules targeting eukaryotic gene sequences, decoy molecules targeting transcription factors [39,40]. Recently, PNAs have been demonstrated to be a very efficient tool to alter the biological functions of microRNAs. This has been firmly demonstrated in studies conducted both *in vitro* and *in vivo* [41-48].

Concerning PNA-based targeting of miRNAs involved in the post-transcriptional regulation of the expression of *CFTR*, we recently reported that a PNA directed against miR-145-5p inhibits biological functions of this miRNA and up-regulates CFTR expression [49]. This finding was confirmed by another research group in CF tissues [50]. This is relevant, considering the increasing interest in RNA-targeting therapies for CF [51-55].

The objective of this paper is to verify whether down-regulation of miR-101-3p by a PNA molecules could alter/modulate CFTR expression. Specific down-regulation of miR-101-3p was achieved with a PNA carrying a R8 poly-arginine motif to facilitate its uptake inside target cells. We then verified whether this was associated with increased expression of CFTR.

2. Results

2.1. Presence of miR-101-3p binding sites within the CFTR 3'-UTR mRNA sequences

Fig. 1A shows the location of the miR-101-3p binding site within the CFTR 3'-UTR mRNA sequence, obtained by performing the analysis employing RNAhybrid, a tool for finding the minimum free energy hybridization of a short sequence RNA to the best fitting part of a long one [49]. This tool is frequently used for microRNA target prediction (https://bibiserv2.cebitec.uni-bielefeld.de/rnahybrid?id=rnahybrid_view_submission). The extent of the interaction between the *CFTR* 3'-UTR mRNA sequence and mature miR-101-3p involves 12 nucleotides (Fig. 1A), located between nucleotide positions 1508 and 1514 of the 3'-UTR *CFTR* mRNA and spatially described in Fig. 1B. The involvement of miR-101-3p in *CFTR* regulation is well known and was previously demonstrated by Viart et al. [11], Hassan et al. [7] and Megiorni et al. [13] using reported luciferase plasmids linked to the 3'-UTR of the *CFTR* mRNA.

2.2. PNAs targeting miR-101-3p

The PNAs utilized in this study (Fig. 1C) were designed according to our standardized protocols [56] based on the following criteria: (a) a short 18-base-pairs length allowing efficient synthesis also on large scale; (b) lack of self-complementarity, both in antiparallel and parallel orientation; (c) minimal length of complementary sequences in mRNAs, as evaluated by BLAST search within the full transcriptome; (d) when possible, targeting the "seed region", which is an essential element for miRNA function. A carrier octarginine R8 peptide was conjugated at the N-terminus of the PNA chain to increase the delivery efficiency, that allows the uptake of R8-PNA in about 100% of the cells, as

elsewhere published [44,46]; this conjugation is easily realized during PNA solid-phase synthesis using the same reagents and solvents.

Concerning efficiency and specificity of PNA-miRNA interactions, we like to underline that (a) only PNAs with strong self-complementarity could fail to bind their target mRNAs, and (b) the efficiency of binding of PNAs with target RNAs is so high that they are even able to disrupt RNA secondary structures. Therefore, rather than efficiency of PNA/RNA interaction, we focused on sequence-specificity.

To this aim, a control PNA (R8-PNA-a101-MUT) was obtained by scrambling the position of 4 nucleobases, thus leaving the same base composition of the two PNAs. Mutated sequences were also analyzed using BLAST search to assess possible interactions with undesired mRNAs. As reported in Fig. 1C (showing the sequences of PNA selected in the present study) R8-PNA-a101-5p displays a fully complementary sequence with the miR-101-3p, while the R8-PNA-a101-MUT contains four scrambled changes, that are known to suppress the hybridization, as shown by circular dichroism and analysis of melting temperatures [57], by arrested RT-qPCR [58] and by SPR-based biospecific interaction analysis (BIA) [59]. As reported in several manuscripts, like PNAs and PNA-DNA chimeras, these R8-PNA-based molecules are resistant to degradation not only in cellular systems *in vitro*, but also in several biological fluids analyzed *in vivo* [60-63].

2.3. R8-PNA-a101 inhibits miR-101-3p

In the experiment depicted in Fig.2A, human airway epithelial Calu-3 cells [64] were cultured for 72 hours in the presence of increasing amounts of R8-PNA-a101 (0.5, 1, 2 and 4 μM). The cells were then harvested, RNA isolated and RT-qPCR performed using primers amplifying miR-101-3p. The results obtained indicate an inhibitory effect on miR-

101-3p specific hybridization when 2 and 4 μM concentrations have been utilized (Fig.2A). Starting from this piece of information, 4 μM concentration was chosen and its inhibitory activity on miR-101-3p demonstrated to be statistically significant in four independent treatments of Calu-3 cells for 72 hours (Fig.2B). These experiments support the concept that treatment of Calu-3 cells with the R8-PNA-a101 leads to miR-101-3p inhibition.

In order to verify sequence-specificity of this effect the scrambled mutant R8-PNA-a101-MUT was employed (see Fig.1C for sequence and comparison with the R8-PNA-a101). When Calu-3 cells were treated with the R8-PNA-a101 and the R8-PNA-a101-MUT molecules (both administered for 72 hours at 4 μM concentration), a clear-cut result was obtained and depicted in Figure 2C. While treatment of Calu-3 cells with R8-PNA-a101 leads to a sharp inhibition of miR-101-3p RT-qPCR signals (black box), as also reported in Fig.2B, the mutant R8-PNA-a101-MUT displayed no inhibitory effects (gray box).

A further representative experiment confirming sequence-specificity of the effects is reported in Supplementary Figure S1. The RT-qPCR signals for other miRNAs expressed in Calu-3 cells (i.e. miR-335-5p) were unchanged following 72 hours treatment with 4 μM R8-PNA-a101, supporting the conclusion that the effect of R8-PNA-a101 is fairly specific. Conversely, preferential inhibition of miR-335-5p was obtained following treatments of Calu-3 cells with a PNA targeting miR-335-5p (R8-PNA-a335, 4 μM). Altogether, the experiments shown in Fig.2C and supplementary Fig.S1 support the conclusion that the effects of R8-PNA-a101 on miR-101-3p are sequence-specific.

2.4. Effects of the R8-PNA-a101 on CFTR mRNA and protein

When Calu-3 cells were cultured in the presence of 4 μM R8-PNA-a101 for 72 hours, a clear effect was observed on *CFTR* gene expression, after RNA and protein isolation,

following analysis using RT-qPCR and Western Blotting approaches. In fact, *CFTR* gene expression was found to be sharply increased (Figure 3, A-C) when analysis was performed using RT-qPCR (Figure 2A) and Western blotting (Figure 3, B and C). Figure 3A shows the increase of *CFTR* mRNA content when Calu-3 cells were treated with the R8-PNA-a101 (but not with R8-PNA-a 101-MUT). The Western blotting experiments (Figure 2, B and C) indicate that the R8-PNA-a101 is efficient in causing an increase of CFTR protein, in agreement with the RT-qPCR data shown in panel A of the same Figure. We previously observed that PNAs against miR-145 and miR-494 were able to increase CFTR protein expression by 1.5-2 folds with respect to the constitutive levels [49]. In this respect, the R8-PNA-a101 seems definitely more potent than R8-PNA-a145 and other PNAs targeting microRNAs miR-433, miR-494 and miR-509, being the induced CFTR/β-actin ratio 3-fold higher than the constitutive expression (Figure 3D). The results reported in Figure S2 (Supplementary data) demonstrate that the PNA tested in these experiments did not exhibit antiproliferative effects and did not reduce the extent of viable Calu-3 cells. Concerning the effect on apoptosis, the PNA retained only a limited pro-apoptotic activity when compared to the validated pro-apoptotic compound Stattic (Selective STAT3 inhibitor).

2.5. R8-PNA-a101 treated Calu-3 cells: miRNome profile studied by next-generation sequencing (NGS)

Despite the fact that treatment of Calu-3 with R8-PNA-a101 leads to a sharp inhibition of miR-101-3p (Figure 2, A and B) and a lower effect on other miRNAs (i.e. miR-335, see Figure S1), we were interested in verifying the effects of PNA treatment on the overall miRNome, in order to obtain information on the specificity of the effects of the treatment. To this aim RNA extracted from Calu-3 cells, either untreated or treated with the

R8-PNA-a101 for 48 hours was analyzed by NGS, using the Illumina NextSeq500 platform and NextSeq® 500/550 High Output Kit v2 (see the Materials and methods section). By the small RNA-Seq approach thousands of small RNA and miRNA sequences can be analyzed with unprecedented sensitivity and dynamic range. In particular, differential expression of all small RNAs in any sample can be measured and variations can be characterized without prior sequence or secondary structure information.

Fig. 4 shows some of the results obtained (the differentially expressed miRNAs are shown in panel A). This dataset is extensively reported and analyzed in an associated paper, containing also all the raw data obtained. By analyzing the NGS data imposing a threshold of 2 FC (Fold Change) value most of the miRNA (403/479, 84.13%) exhibited changes below this threshold value. On the contrary 19 miRNAs (3.97%) were found to be up-regulated (12 miRNAs with a FC between 2 and 3, 7 miRNAs with a FC >3), and 57 miRNAs (11.90%) were found down-regulated (35 miRNAs with a FC between 2 and 3, 22 miRNAs with a FC >3) (Figure 4B and Table III). The miRNA list derived from the NGS data and shown in Table III was compared with (a) the list of miRNAs dysregulated in cystic fibrosis (Table II), (b) the list of miRNAs putatively able to target the 3'-UTR sequences of the CFTR mRNA and mRNAs coding for CFTR positive regulators (such as NHERF1, NHERF2, ezrin) (Table I) [16-19]. This comparison showed that only two miRNAs were found to be present in all the lists, miR-155-5p and miR-125b-5p (Figure 4C), while down-regulated miR-132-3p and miR-6873-3p were present also in the miRNAs targeting CFTR and CFTR regulators. Notably, miR-125b-5, miR-155-5p and miR-6873-3p targets NHERF2 (a CFTR regulator) [14,15] and miR-132-3p has been proposed to target CFTR, similarly to miR-101-3p [7,11-15]. In addition, miR-155-5p deserves further consideration, since it has been demonstrated to be involved in cystic fibrosis by regulating FOXO1 [24]; it is expected to regulate TCF4 [65], a transcription factor positively affecting CFTR expression [66]; and (d) promotes inflammatory responses following miR-

155 dependent hyperactivation of IL-8 [25]. In consideration of these observations, an RT-qPCR analysis was therefore conducted analyzing the effects of R8-PNA-a101 treatment on miR-155-5p and miR-125b-5p, fully confirming the NGS results (Figure 5). These data indicate that the inhibitory activity of a single PNA on the specific miRNA (in our case miR-101-3p), is accompanied by changes in the expression levels of other microRNAs (for instance miR-155-5p and miR-125-5p), which might be involved in the regulation of the same pathway(s) (in our case CFTR expression).

3. Discussion

Since the identification of the (miRNAs) involved in the regulation of *CFTR* gene [6-11], miRNAs known to down-regulate the expression of the CFTR and associated proteins have been investigated as potential therapeutic targets. For instance, PNA-mediated inhibition of miR-145-5p (which down-regulates CFTR) leads to CFTR increase in the Calu-3 model system [49].

The results here described show that miR-101-3p, targeting the 3'-UTR sequence of the *CFTR* mRNA [7,11,13] can be selectively inhibited by a PNA carrying a full complementary sequence. With respect to clinical relevance of microRNA targeting, it is expected that decreased availability of miRNAs (anti-miRNA approach) is associated with an accumulation of target mRNAs and/or increase of the encoded protein; conversely, increased expression of miRNAs (miRNA mimicking/replacement strategy) is expected to decrease the expression of the target mRNAs [49]. In this context Peptide Nucleic Acids [34] represent molecules with antimiRNA activity [44-49]. Our data sustain this concept, demonstrating up-regulation of CFTR following PNA-mediated inhibition of miR-101-3p (Figure 3).

While the results here presented are a strong proof-of-principle that PNA-based targeting miRNAs regulating CFTR might lead to CFTR up-regulation, several considerations should be discussed. First of all, as for other mRNAs, CFTR is regulated by several miRNAs [14,15]. Therefore, targeting other miRNAs might lead to even more efficient up-regulation of CFTR. Second, possible combined effects of PNAs targeting different miRNAs involved in CFTR regulation should be in the future considered in order to obtain the highest level of CFTR induction. Third, our observations should be confirmed in CF cellular model systems.

Another important conclusion derived from the results here presented is that the miRNome is to some extend affected by the treatment of target cells with anti-miRNA PNAs. Despite the fact that the majority of miRNA (more than 84%) display low FC values after R8-PNA-a101 treatment of Calu-3 cells (FC < 2), the results depicted in Figure 4 show that some miRNAs are up-regulated and some miRNAs are down-regulated following this treatment. Interestingly, most of the PNA-dysregulated miRNAs are different when different PNAs are employed. For instance, when we compare the list of miRNAs displaying FC>3 in cells treated with R8-PNA-a101 (Figure 4) and R8-PNA-a145 [67], only 6 miRNAs were found in common, while the majority (24 for R8-PNA-a101 and 18 for R8-PNA-a145) were found specifically dysregulated for each PNA-based treatment. This finding supports the concept of a sequence-selectivity of the PNA-treatments targeting specific microRNAs. This is extensively discussed in the companion paper by Gasparello et al. [68].

While the miRNAs specifically modulated by the R8-PNA-a101 are only a large minority of the overall Calu-3 expressed miRNAs detectable by the NGS approach, our data sustains the concept that selective miRNA inhibition (in our case inhibition of miR-101-3p) might be accompanied by co-inhibition of other miRNAs (for instance miR-155-5p and miR-125b-5p). This might be of great interest in using one PNA molecule affecting different miRNAs, either directly (miR-101-3p) or indirectly (miR-155-5p and miR-125b-5p, see Figure 5). This modulation deserves further studies, since all these miRNAs are involved in Cystic Fibrosis.

While the mechanism of action of miR-101-3p on miR-155-5p and miR-125b-5p expression remains to be elucidated, we would like to comment that miR-miR-155-3p is responsible for hyperactivating IL-8, thereby promoting CF inflammation [25]. Therefore, PNA-mediated targeting of miR-101-3p in Calu-3 cells is expected to lead, in addition to up-regulation of CFTR (Figure 3) down-regulation of IL-8, possibly combining two activities

of great interest in CF therapy, i.e. CFTR modulation and anti-inflammatory effects. Considering the effect of the R8-PNA-a101 on miR-155-5p and miR-125b-5p, two additional mechanisms can be hypothesized (summarized in Figure 6): (a) an up-regulation of NHERF2, mediated by the decrease of miR-155-5p and miR-125b-5p [14,15] and (b) an up-regulation of TCF4 [65] (caused by a decrease of miR-155-5p), which, in addition to CFTR up-regulation [66], is a potent repressor of another CFTR-regulating miRNA, miR-145 [69]. In this specific case, down regulation of miR-145 might lead to increased expression of CFTR as recently published by our research group, using a PNA targeting miR-145 [49] (see Figure 6).

Despite the fact that the hypothesis depicted in Figure 6 should be experimentally validated in each presented step, this study is the proof-of-principle that miRNA targeting might increase CFTR expression. In this regulatory network the participation of miRNAs/transcription factors interactions appears to be of great interest and impact, deserving further studies.

With respect to a possible miRNA-based therapeutic option in CF, our results are of interest for the personalized therapy of CF, which is characterized by more than 300 CFTR disease-causing mutations (www.genet.sickkids.on.ca/cftr/) [70]. In consideration of the different mutations of the *CFTR* gene, targeting miR-101-3p could be useful for type IV (less function), V (less protein) and VI (less stable protein) CFTR defect. For CFTR defects such as type I (no protein), II (no traffic), III (no function) miR-101-3p targeting may be however considered in combination with other therapeutic protocols based on the use of CFTR correctors [70-73].

4. Material and Methods

Synthesis and Characterization of PNAs. The synthesis and characterization of anti-miR-101 PNAs were similar to those previously reported by Brognara et al. [46]. The synthesis was performed utilizing a standard Fmoc-based automated peptide synthesizer (Syro I, MultiSynTech GmbH, Witten, Germany), using a ChemMatrix-RinkAmide resin loaded with Fmoc-Gly-OH (0.2 mmol/g) as first monomer and commercially available monomers (Link Technologies, Bellshill, UK) with HBTU/DIPEA coupling. Efficient cleavage from the solid support was obtained with 10% m-cresol in trifluoroacetic acid, followed by precipitation and washings with diethyl ether. Purification was performed by HPLC using a Phenomenex Jupiter RPC1, (250- 4.6mm, 1.7µm) column. Gradient: 100% A for 5 minutes, then from 0% to 50% B in 30 minutes at 0.25 mL/min flow (A: water + 0.1% trifluoroacetic acid; B: acetonitrile + 0.1% trifluoroacetic acid). After purification the PNAs identity and purity were confirmed by UPLC/ESI-MS, (Waters Acquity ultra performance LC HO6UPS-823M, with Waters SQ detector equipped with Waters UPLC BEH C18, 50×2.1 mm, 1.7 µm) at 35°C. A flow rate of 0.25 ml/min was used with the following solvent systems: (A): 0.2% FA in H_2O and (B): 0.2% FA in MeCN (FA = formic acid). The column was flushed for 0.9 min with solvent A, then a gradient from 0 to 50% B in 5.7 min was used.

The concentration of the PNA was calculated using UV-absorbance at 260 nm assuming an additive contribution of nucleobases.

The syntheses and characterizations of R8-PNA-a145, R8-PNA-a509, R8-PNA-a494, R8-PNA-a433 were reported elsewhere [49]. The UPLC-ESI/MS analysis of R8-PNA-a101 and R8-PNA-a101-MUT, and the characterization of R8-PNA-a335 are reported in Supplementary data (Fig.S4 and Fig.S5, respectively).

R8-PNA-a101: sequence H-R_8-AGTTATCACAGTACTGTA-Gly-NH_2 UPLC/ESI-MS R_t= 2.67 min, *calculated MW*: 6200.34 g/mol; *m/z found (calculated)*: 1241.0 (1241.07) $[MH_5]^{5+}$, 1034.4 (1034.39) $[MH_6]^{6+}$, 886.8 (886.73) $[MH_7]^{7+}$, 776.0 (776.04) $[MH_8]^{8+}$, 690.0 (689.93) $[MH_9]^{9+}$, 621.0 (621.03) $[MH_{10}]^{10+}$.

R8-PNA-a101-MUT: sequence H-R8-AGTAATCTCAGGACTTTA-Gly-NH_2 (mutations underlined); UPLC/ESI-MS R_t= 2.76 min, *calculated MW*: 6200.34 g/mol; *m/z found (calculated)*: 1240.89 (1241.07) $[MH_5]^{5+}$, 1034.24 (1034.39) $[MH_6]^{6+}$, 886.73 (886.73) $[MH_7]^{7+}$, 775.88 (776.04) $[MH_8]^{8+}$, 689.94 (689.93) $[MH_9]^{9+}$, 621.02 (621.03) $[MH_{10}]^{10+}$.

Culture Conditions. Calu-3 cells [55] were cultured in humidified atmosphere of 5% CO_2/air in DMEM/F12 medium (Gibco, Grand Island, US) supplemented with 10% fetal bovine serum (Biowest, Nauillè, Francia), 100 units/ml penicillin and 100 mg/mL streptomycin (Lonza, Verviers, Belgio) and 1% NEEA (100X) (Non-Essential Amino Acids Solution; Gibco). To determine the effect on proliferation, cell growth was monitored by determining the cell number/ml using a Z2 Coulter Counter (Coulter Electronics, Hialeah, FL, USA).

RNA Extraction. Calu-3 cells were trypsinized and collected by centrifugation at 1,500 rpm for 10 minutes at 4°C. The cellular pellets were washed twice with PBS and lysed with the Tri-Reagent (Sigma Aldrich, St. Louis, Missouri, USA). RNA extraction was conducted following the manufacturer's instructions and the isolated RNA was washed once with cold 75% ethanol. After this washing step, the RNA was dried and dissolved in nuclease free pure water and considered ready for the use.

Quantitative Analyses of miRNAs. For miRNA quantification using real-time RT-qPCR reagents, the primers and probes (Assay name:hsa-miR-335-5p, TM:000546; Assay name:hsa-miR-101-3p, TM:002253; Assay name:hsa-miR-125b-5p, TM:000449; Assay name:hsa-miR-155-5p, TM:002623) were obtained from Applied Biosystems. The TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) was

employed for Reverse transcriptase (RT) reactions; real-time PCR was performed according to the manufacturer's protocols. Each assay was performed using 300 ng sample and all the RT-PCR reactions were performed using the CFX96 Touch Real-Time PCR Detection System (BioRad, Hercules, CA, USA). The U6 snRNA reference (TM:001973) was employed to normalize all RNA samples, as previously reported [44,46,49].

Analysis of CFTR expression: RT-qPCR. Gene expression analysis was performed by RT-qPCR. 300 ng of the total RNA was reverse transcribed by using random hexamers. Quantitative real-time PCR (qPCR) assays were carried out using gene-specific double fluorescently labeled probes. Primers and probes used to assay CFTR (Assay ID: Hs00357011_m1) gene expression were purchased from Applied Biosystems (Applied Biosystems, Foster City, CA, USA). The relative expression was calculated using the comparative cycle threshold method and, as reference genes, the human RPL13A (Assay ID: Hs03043885_g1) [49].

Analysis of CFTR expression: Western blotting. Cell pellets were lysed as reported by Fabbri et al. [49]. For CFTR analysis 20 μg of total protein was heated in Laemmli buffer (Bio-Rad Laboratories, Hercules, California, USA) and loaded onto a 3 to 8% Tris-acetate gel (Bio-Rad Laboratories, Hercules, California, USA). The gel was transferred to PVDF membrane by using Trans Blot Turbo (Bio-Rad Laboratories, Hercules, California, USA). For CFTR detection and quantification, the mouse monoclonal antibody, clone 596, against NBD2 domain of CFTR (University of North Carolina, Cystic Fibrosis Center, Chapel Hill, NC) was employed at a dilution of 1:2500. After washes, membranes were incubated with horseradish peroxidase-coupled anti-mouse immunoglobulin (R&D System, Minneapolis, MN, USA) at room temperature for 1 hour and after washes the signal was developed by enhanced chemiluminescence (LumiGlo Reagent and Peroxide, Cell Signaling). After membranes stripping, β-Actin monoclonal

antibody (Sigma-Aldrich, St. Louis, Mo, USA) was used as a standard internal control [74-76].

Next Generation Sequencing (RNA-Seq). NGS experiments were performed at the Laboratory for Technologies of Advanced Therapies (LTTA) of Ferrara University. SmallRNA libraries were prepared from total RNA using TruSeq® Small RNA Library PrepKit v2 (Illumina, RS-200-0012/24/36/48), according to manufacturer's indications. Briefly, 35 ng of purified RNA were linked to RNA 3' and 5' adapters, converted in cDNA and amplified using Illumina primers containing unique indexes for each sample. Libraries were quantified through Agilent Bioanalyzer, using High Sensitivity DNA kit (Agilent, 5067-4626); after size-selection and ethanol precipitation, the library pool was quantified through Agilent Bioanalyzer and High Sensitivity DNA kit, denatured and diluted to 1.8 pM and sequenced using Illumina NextSeq500 platform and NextSeq® 500/550 High Output Kit v2 (75 cycles) (Illumina, FC-404-2005). Raw base-call data generated from the Illumina NextSeq 500 system have been demultiplexed and converted to FASTQ format. After quality check, evaluated using FastQC tool (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), adapters sequences have been trimmed by Cutadapt (http://cutadapt.readthedocs.io/en/stable/index.html). In this step also sequences shorter than 10 nucleotides have been removed. Reads mapping has been performed using the STAR algorithm (https://www.ncbi.nlm.nih.gov/pubmed/23104886), and the reference genome was composed of human microRNAs sequences from the miRbase 22 (http://www.mirbase.org/). Count of raw mapped reads has been performed using the htseq-count script from the HTSeq tools (http://www-huber.embl.de/HTSeq/doc/overview.html); raw counts have been normalized using DESeq2 bioconductor package (http://bioconductor.org/packages/release/bioc/html/DESeq2.html). The employed softwares use the Benjamini–Hochberg method to control the False Discovery Rate (FDR). The optimal read depth to analyse the miRNA transcriptome was determined at 10 million

reads per sample. Differentially expressed miRNAs were employed for Cluster Analysis of samples using the Manhattan correlation as a measure of similarity [68].

Statistical Analysis. In order to detect significance of the observed effects, results have been expressed as mean ± standard deviation (S.D.) and comparison among groups was made by using analysis of variances (ANOVA) with Dunnett's test for comparison with a single control. Statistical significance was defined as significant (*, $p < 0.05$) and highly significant (**, $p < 0.01$).

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Appendix A. Supplementary data

Supplementary data to this article can be found online. All the raw data related to NGS can be found in the companion dataset paper by Gasparello et al. [68] and at the public expression database "European Nucleotide Archive" (https://www.ebi.ac.uk/ena) with accession number PRJEB39141 (https://www.ebi.ac.uk/ena/browser/view/PRJEB39141).

Author Contributions

R.G., R.C., M.C.D. and G.C. conceived and designed the experiments; E.F. and A.F. designed and analyzed the biological experiments; T.J. and A.M. performed the synthesis and purification of the PNA molecules; E.F., G.S., M.B. and J.G. performed the RT-qPCR

based experiments; E.F. and I.L. performed the experiments on apoptosis; A.T. and S.M. performed the Western blotting; A.M. performed CD, UV and MS experiments; E.F., R.G., R.C., M.C.D. and G.C. wrote the paper.

Conflicts of Interest

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

Abbreviations

CF	Cystic fibrosis
CFTR (CFTR)	Cystic Fibrosis Transmembrane Conductance Regulator
FA	Formic acid
FC	Fold content
FOXO1	Forkhead Box O1
HPLC	High performance liquid chromatography
UPLC	Ultra performance liquid chromatography
ESI-MS	Electrospray Ionisation Mass Spectrometry
IL-8	Interleukin-8
miRNA	MicroRNA
NEEA	Non-Essential Amino Acids Solution
NGS	Next Generation Sequencing
NHERF1	Na(+)/H(+) Exchange Regulatory Factor 1
NHERF2	Na(+)/H(+) Exchange Regulatory Factor 2
PBS	Phosphate buffered saline
PNA	Peptide nucleic acid
PVDF	Polyvinylidene fluoride
RISC	RNA-induced silencing complex

RT-PCR	Reverse transcription polymerase-chain reaction
SDS	Sodium dodecylsulphate
SDS-PAGE	SDS-polyacrylamide-gel electrophoresis
STAT	Signal Transducer And Activator Of Transcription
UTR	Untranslated region

References

1. E.J. Sontheimer, R.W. Carthew, Silence from within: endogenous siRNAs and miRNAs, Cell 122 (2005) 9-12. https://doi.org/10.1016/j.cell.2005.06.030.

2. I. Alvarez-Garcia, E.A Miska, MicroRNA functions in animal development and human disease, Development 132 (2005) 4653-4662. https://doi.org/ 10.1242/dev.02073.

3. L. He, G.J.Hannon, MicroRNAs: small RNAs with a big role in gene regulation, Nat Rev Genet. 5 (2004) 22-531. https://doi.org/ 10.1038/nrg1379.

4. L.P. Lim, N.C. Lau, Garrett-Engele P, A. Grimson, J.M. Schelter, J. Castle, D.P. Bartel, P.S. Linsley, J.M. Johnson, Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs, Nature 433 (2005) 769-773. https://doi.org/ 10.1038/nature03315.

5. B.P. O'Sullivan, S.D. Freedman, Cystic fibrosis, Lancet 373 (2009) 1891-1904. https://doi.org/10.1016/s0140-6736(09)60327-5.

6. A.E. Gillen, N. Gosalia, S.H. Leir, A. Harris, MicroRNA regulation of expression of the cystic fibrosis transmembrane conductance regulator gene, Biochem J. 438 (2011) 25-32. https://doi.org/ 10.1042/BJ20110672.

7. F. Hassan, G.J. Nuovo, M. Crawford M, P.N. Boyaka, S. Kirkby, S.P. Nana-Sinkam, E. Cormet-Boyaka, MiR-101 and miR-144 regulate the expression of the CFTR chloride channel in the lung, PLoS One 7 (2012) e50837. https://doi.org/ plos.org/plosone/article?id=10.1371/journal.pone.0050837

8. S. Ramachandran, P.H.Karp, P. Jiang, L.S. Ostedgaard, A.E. Walz, J.T. Fisher, S. Keshavjee, K.A. Lennox, A.M. Jacobi, S.D. Rose, M.A. Behlke, M.J. Welsh, Y. Xing, P.B. McCray, A microRNA network regulates expression and biosynthesis of wild-type and DeltaF508 mutant cystic fibrosis transmembrane conductance regulator, Proc Natl Acad

Sci USA 109 (2012) 13362-13367. https://doi.org/ 10.1073/pnas.1210906109.

9. S. Ramachandran, P.H. Karp, S.R. Osterhaus, P. Jiang, C. Wohlford-Lenane, K.A. Lennox, A.M. Jacobi, K. Praekh, S.D. Rose, M.A. Behlke, Y. Xing, M.J. Welsh, P.B. McCray, Post-transcriptional regulation of cystic fibrosis transmembrane conductance regulator expression and function by microRNAs, Am J Respir Cell Mol Biol. 49 (2013) 544-551. https://doi.org/ 10.1165/rcmb.2012-0430OC.

10. I.K. Oglesby, S.H. Chotirmall, N.G. McElvaney, C.M. Greene, Regulation of cystic fibrosis transmembrane conductance regulator by microRNA-145, -223, and -494 is altered in ΔF508 cystic fibrosis airway epithelium. J Immunol. 190 (2013) 3354-3362. https://doi.org/ 10.4049/jimmunol.1202960.

11. V. Viart, A. Bergougnoux, J. Bonini, J. Varilh, R. Chiron, O. Tabary, N. Molinari, M. Claustres, M. Taulan-Cadars, Transcription Factors and miRNAs That Regulate Fetal to Adult CFTR Expression Change Are New Targets for Cystic Fibrosis. Eur Respir J. 45 (2015) 116-128. https://doi.org/10.1183/09031936.00113214.

12. P.J. McKiernan, C.M. Greene, MicroRNA Dysregulation in Cystic Fibrosis, Mediators of Inflammation 2015 (2015) 529642. https://doi.org/ 10.1155/2015/529642.

13. F. Megiorni, S. Cialfi, C. Dominici, S. Quattrucci, A. Pizzuti, Synergistic post-transcriptional regulation of the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) by miR-101 and miR-494 specific binding, PLoS One 6 (2011) e26601. https://doi.org/ 10.1371/journal.pone.0026601.

14. C.H. Chou, S. Shrestha, C.D. Yang, et al. miRTarBase update 2018: a resource for experimentally validated microRNA-target interactions. Nucleic Acids Res. 46 (2018) D296-D302. https://doi.org/ 10.1093/nar/gkx1067.

15. H.Y. Huang, Y.C. Lin, J. Li, K.Y. Huang, S. Shrestha, H.C. Hong, Y. Tang, Y.G. Chen, C.N. Jin, Y. Yu, J.T. Xu, Y.M. Li, X.X. Cai, Z.Y. Zhou, X.H. Chen, Y.Y. Pei, L. Hu, J.J. Su, S.D. Cui, F. Wang, Y.Y. Xie, S.Y. Ding, M.F. Luo, C.H. Chou, N.W. Chang, K.W. Chen, Y.H. Cheng, X.H. Wan, W.L. Hsu, T.Y. Lee, F.X. Wei, H.D. Huang, miRTarBase 2020:

updates to the experimentally validated microRNA-target interaction database. Nucleic Acids Res. 48 (2020) D148-D154. https://doi.org/10.1093/nar/gkz896.

16. N. Sharma, J. LaRusch, P.R. Sosnay, L.B. Gottschalk, A.P. Lopez, M.J. Pellicore, T. Evans, E. Davis, M. Atalar, C.H. Na, G.D. Rosson, D. Belchis, M. Milewski, A. Pandey, G.R. Cutting, A sequence upstream of canonical PDZ-binding motif within CFTR COOH-terminus enhances NHERF1 interaction. Am J Physiol Lung Cell Mol Physiol. 311 (2016) L1170-L1182. https://doi.org/10.1152/ajplung.00363.2016.

17. J. Holcomb, Y. Jiang, G. Lu, L. Trescott, J. Brunzelle, N. Sirinupong, C. Li, A.P. Naren, Z. Yang, Structural insights into PDZ-mediated interaction of NHERF2 and LPA(2), a cellular event implicated in CFTR channel regulation. Biochem Biophys Res Commun. 446 (2014) 399-403. https://doi.org/10.1016/j.bbrc.2014.02.128.

18. A.C. Abbattiscianni, M. Favia, M.T. Mancini, R.A. Cardone, L. Guerra, S. Monterisi, S. Castellani, O. Laselva, F. Di Sole, M. Conese, M. Zaccolo, V. Casavola, Correctors of mutant CFTR enhance subcortical cAMP-PKA signaling through modulating ezrin phosphorylation and cytoskeleton organization. J Cell Sci. 129 (2016) 1128-1140. https://doi.org/10.1242/jcs.177907.

19. D. Reczek, A. Bretscher, The carboxyl-terminal region of EBP50 binds to a site in the aminoterminal domain of ezrin that is masked in the dormant molecule. J Biol Chem. 273 (1998) 18452-18458. https://doi.org/10.1074/jbc.273.29.18452.

20. M.L. Endale Ahanda, T. Bienvenu, I. Sermet-Gaudelus, L. Mazzolini, A. Edelman, R. Zoorob, N. Davezac, The hsa-miR-125a/hsa-let-7e/hsa-miR-99b Cluster Is Potentially Implicated in Cystic Fibrosis Pathogenesis. J Cyst Fibros. 14 (2014) 571-579. https://doi.org/10.1016/j.jcf.2015.02.011.

21. I.K. Oglesby, I.M. Bray, S.H. Chotirmall, R.L. Stallings, S.J. O'Neill, N.G. McElvaney, C.M. Greene, miR-126 Is Downregulated in Cystic Fibrosis Airway Epithelial Cells and Regulates TOM1 Expression. J Immunol. 184 (2010) 1702-1709. https://doi.org/10.4049/jimmunol.0902669.

22. L.R. Stolzenburg, S. Wachtel, H. Dang, A. Harris, miR-1343 attenuates pathways of fibrosis by targeting the TGF-β receptors. Biochem J. 473 (2016) 245-256. https://doi.org/10.1042/BJ20150821.

23. A. Kivihall, A. Aab, J. Soja, K. Sładek, M. Sanak, A. Altraja, B. Jakiela, G. Bochenek, A. Rebane, Reduced expression of miR-146a in human bronchial epithelial cells alters neutrophil migration. Clinical and Translational Allergy. 9 (2019) 62. https://doi.org/10.1186/s13601-019-0301-8.

24. L. Montanini, A. Smerieri, M. Gullì, F. Cirillo, G. Pisi, C. Sartori, S. Amarri, S. Bernasconi, N. Marmiroli, M.E. Street, miR-146a, miR-155, miR-370, and miR-708 Are CFTR-Dependent, Predicted FOXO1 Regulators and Change at Onset of CFRDs. The Journal of Clinical Endocrinology & Metabolism. 101 (2016) 4955-4963. https://doi.org/10.1210/jc.2016-2431.

25. S. Bhattacharyya, N.S. Balakathiresan, C. Dalgard, U. Gutti, D.Armistead, C. Jozwik, M. Srivastava, H.B. Pollard, R. Biswas, Elevated miR-155 Promotes Inflammation in Cystic Fibrosis by Driving Hyperexpression of Interleukin-8. J Biol Chem. 286 (2011) 11604–11615. https://doi.org/ 10.1074/jbc.M110.198390.

26. P. Kumar, S. Bhattacharyya, K.W. Peters, M.L. Glover, A. Sen, R.T. Cox, S. Kundu, H. Caohuy, R.A. Frizzell, H.B. Pollard, R. Biswas, miR-16 rescues F508del-CFTR function in native cystic fibrosis epithelial cells. Gene Therapy. 22 (2015) 908–916. https://doi.org/10.1038/gt.2015.56.

27. I.K. Oglesby, S.F. Vencken, R. Agrawal, K. Gaughan, K. Molloy, G. Higgins, P. McNally, N.G. McElvaney, M.A. Mall, C.M. Greene. miR-17 Overexpression in Cystic Fibrosis Airway Epithelial Cells Decreases interleukin-8 Production. Eur Respir J. 46 (2015) 1350-1360. https://doi.org/10.1183/09031936.00163414.

28. A.M. Pierdomenico, S. Patruno, M. Codagnone, F. Simiele, V.C. Mari, R. Plebani, A. Recchiuti, M. Romano, microRNA-181b is increased in cystic fibrosis cells and impairs lipoxin A4 receptor-dependent mechanisms of inflammation resolution and antimicrobial defense. Scientific Reports. 7 (2017) 13519. https://doi.org/10.1038/s41598-017-14055-y.

29. Y. Fan, Y. Zhou, X. Zhou, F. Sun, B. Gao, M. Wan, X. Zhou, J. Sun, X. Xu, L. Cheng, J. Crane, L. Zheng, MicroRNA 224 Regulates Ion Transporter Expression in Ameloblasts To Coordinate Enamel Mineralization. Mol Cell Biol. 35 (2015) 2875-2890. https://doi.org/10.1128/MCB.01266-14.

30. S. Weldon, P. McNally, D.F. McAuley, I.K. Oglesby, C.L. Wohlford-Lenane, J.A. Bartlett, C.J. Scott, N.G. McElvaney, C.M. Greene, P.B. McCray, C.C. Taggart, miR-31 Dysregulation in Cystic Fibrosis Airways Contributes to Increased Pulmonary Cathepsin S Production. Am J Respir Crit Care Med. 190 (2014) 165-174. https://doi.org/10.1164/rccm.201311-1986OC.

31. F. Amato, R. Tomaiuolo, F. Nici, N. Borbone, A. Elce, B. Catalanotti, S. D'Errico, C.M. Morgillo, G. De Rosa, L. Mayol, G. Piccialli, G. Oliviero, Exploitation of a Very Small Peptide Nucleic Acid as a New Inhibitor of miR-509-3p Involved in the Regulation of Cystic Fibrosis Disease-Gene Expression. Biomed Res Int. 2014 (2014) 610718. https://doi.org/10.1155/2014/610718.

32. E. Fabbri, M. Borgatti, G. Montagner, N. Bianchi, A. Finotti, I. Lampronti, V. Bezzerri, M.C. Dechecchi, G. Cabrini, R. Gambari, Expression of microRNA-93 and Interleukin-8 during Pseudomonas aeruginosa-mediated induction of proinflammatory responses. Am J Respir Cell Mol Biol. 50 (2014) 1144-1155. https://doi.org/10.1165/rcmb.2013-0160OC.

33. F. Sonneville, M. Ruffin, C. Coraux, N. Rousselet, P. Le Rouzic, S. Blouquit-Laye, H. Corvol, O. Tabary, MicroRNA-9 downregulates the ANO1 chloride channel and contributes to cystic fibrosis lung pathology. Nat Commun. 8 (2017) 710. https://doi.org/10.1038/s41467-017-00813-z.

34. P.E. Nielsen, M. Egholm, R.H. Berg, O. Buchardt. Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide, Science 254 (1991) 1497-500. https://doi.org/ 10.1126/science.1962210.

35. P.E. Nielsen. Targeting double stranded DNA with peptide nucleic acid (PNA). Curr Med Chem 8 (2001) 545-550. https://doi.org/ 10.2174/0929867003373373.

36. M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S.M. Freier, D.A. Driver, R.H. Berg, S.K. Kim, B. Norden, P.E. Nielsen, PNA hybridizes to complementary oligonucleotides obeying the Watson-Crick hydrogen-bonding rules. Nature 365 (1993) 566-568. https://doi.org/ 10.1038/365566a0.

37. P.E Nielsen, Gene targeting and expression modulation by peptide nucleic acids (PNA), Curr Pharm Des. 16 (2010) 3118-3123. https://doi.org/ 10.2174/138161210793292546.

38. M. Borgatti, I. Lampronti, A. Romanelli, C. Pedone, M. Saviano, N. Bianchi, C. Mischiati, R. Gambari, Transcription factor decoy molecules based on a peptide nucleic acid (PNA)-DNA chimera mimicking Sp1 binding sites. J Biol Chem 278 (2003) 7500-7509. https://doi.org/10.1074/jbc.M206780200.

39. R. Gambari. Peptide-nucleic acids (PNAs): a Tool for the development of gene expression modifiers. Curr Pharm Des. 7 (2001) 1839-1862. https://doi.org/ 10.2174/1381612013397087.

40. R. Gambari. Biological activity and delivery of peptide nucleic acids (PNA)-DNA chimeras for transcription factor decoy (TFD) pharmacotherapy. Curr Med Chem. 11 (2004) 1253-1263. https://doi.org/ 10.2174/0929867043365242.

41. M.M. Fabani, M.J. Gait, MiR-122 targeting with LNA/2'-O-methyl oligonucleotide mixmers, peptide nucleic acids (PNA), and PNA-peptide conjugates, RNA 14 (2008) 336-346. https://doi.org/ 10.1261/rna.844108.

42. M.M. Fabani, C. Abreu-Goodger, D. Williams, P.A. Lyons, P.A. Lyons, A.G. Torres, K.G.C. Smith, A.J. Enright, M.J. Gait, E. Vigorito, Efficient inhibition of miR-155 function in vivo by peptide nucleic acids, Nucleic Acids Res 38 (2010) 4466-4475. https://doi.org/ 10.17863/CAM.15536.

43. P.N. Brown, H. Yin, PNA-based microRNA inhibitors elicit anti-inflammatory effects in microglia cells. Chem Commun (Camb) 49 (2013) 4415-4417. https://doi.org/ 10.1039/C2CC36540E.

44. E. Brognara, E. Fabbri, F. Aimi, A. Manicardi A, N. Bianchi, A. Finotti, G. Breveglieri, M. Borgatti, R. Corradini, R. Marchelli, R. Gambari. Peptide nucleic acids targeting miR-221 modulate p27Kip1 expression in breast cancer MDA-MB-231 cells. Int J Oncol. 41 (2012) 2119-2127. https://doi.org/ 10.3892/ijo.2012.1632.

45. R. Gambari, E. Fabbri, M. Borgatti, I. Lampronti, I. Lampronti, A. Finotti, E. Brognara, N. Bianchi, A. Manicardi, R. Marchelli, R. Corradini, Targeting microRNAs involved in human diseases: a novel approach for modification of gene expression and drug development. Biochem Pharmacol. 82 (2011) 1416-1429. https://doi.org/10.1016/j.bcp.2011.08.007.

46. E. Brognara, E. Fabbri, E, Bazzoli, G. Montagner, C. Ghimenton, A. Eccher, C. Cantù, A. Manicardi, N. Bianchi, A. Finotti, G. Breveglieri, M. Borgatti, R. Corradini, V. Bezzerri, G. Cabrini, R. Gambari, Uptake by human glioma cell lines and biological effects of a peptide-nucleic acids targeting miR-221, J Neurooncol. 118 (2014) 19-28. https://doi.org/ 10.1007/s11060-014-1405-6.

47. E. Fabbri, A. Manicardi, T. Tedeschi, S. Sforza S, N. Bianchi, E. Brognara, A. Finotti, G. Breveglieri, M. Borgatti, R. Corradini, R. Marchelli, R. Gambari, Modulation of the biological activity of microRNA-210 with peptide nucleic acids (PNAs), Chem Med Chem 6 (2011) 2192-2202. https://doi.org/ 10.1002/cmdc.201100270.

48. E. Fabbri, E. Brognara, M. Borgatti, I. Lampronti, A. Finotti, N. Bianchi, S. Sforza, T. Tedeschi, A. Manicardi, R. Marchelli, R. Corradini, R. Gambari. miRNA therapeutics: delivery and biological activity of peptide nucleic acids targeting miRNAs, Epigenomics 3 (2011) 733-745. https://doi.org/ 10.2217/epi.11.90.

49. E. Fabbri, A. Tamanini, T. Jakova, J. Gasparello, A. Manicardi, R. Corradini, G. Sabbioni, A. Finotti, M. Borgatti, I. Lampronti, S. Munari, M.C. Dechecchi, G. Cabrini, R. Gambari, A Peptide Nucleic Acid against MicroRNA miR-145-5p Enhances the Expression of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) in Calu-3 Cells, Molecules, 23 (2017) :E71. https://doi.org/ 10.3390/molecules23010071.

50. F.L. Kabir, N. Ambalavanan, G. Liu, P. Li, G.M. Solomon, C.V. Lal, M. Mazur, B. Halloran, T. Szul, W.T. Gerthoffer, S.M. Rowe, W.T. Harris, MicroRNA-145 Antagonism Reverses TGF-β Inhibition of F508del CFTR Correction in Airway Epithelia. Am J Respir Crit Care Med. 197 (2018) 632-643. https://doi.org/ 10.1164/rccm.201704-0732OC.

51. A. Finotti, J. Gasparello, E. Fabbri, A. Tamanini, R. Corradini, M.C. Dechecchi, G. Cabrini, R. Gambari, Enhancing the Expression of CFTR Using Antisense Molecules Against MicroRNA miR-145-5p, Am J Respir Crit Care Med. 199 (2019) 1443-1444. https://doi.org/10.1164/rccm.201901-0019LE.

52. C.M. Greene, D. Hartl. Developmental control of CFTR: from bioinformatics to novel therapeutic approaches. European Respiratory Journal 45 (2015) 18-20. https://doi.org/ 10.1183/09031936.00138914.

53. P. Bardin, F. Sonneville, H. Corvol, O. Tabary. Emerging microRNA Therapeutic Approaches for Cystic Fibrosis, Front Pharmacol 9 (2018) 1113. https://doi.org/ 10.3389/fphar.2018.01113

54. A.M.A. Glasgow, C. De Santi, C.M. Greene. Non-coding RNA in cystic fibrosis. Biochem Soc Trans. 46 (2018) 619-630. https://doi.org/ 10.1042/BST20170469.

55. B.Q. Shen, W.E. Finkbeiner, J.J. Wine, R.J. Mrsny, J.H. Widdicombe, Calu-3: a human airway epithelial cell line that shows cAMP-dependent Cl- secretion. Am J Physiol. 266 (1994) L493-lL501. https://doi.org/10.1152/ajplung.1994.266.5.L493.

56. A. Manicardi, R. Gambari, L. de Cola, R. Corradini, Preparation of Anti-miR PNAs for Drug Development and Nanomedicine. Methods Mol Biol. 1811 (2018) 49-63. https://doi.org/10.1007/978-1-4939-8582-1_4.

57. S. Croci, A. Manicardi, S. Rubagotti, M. Bonacini, M. Iori, P.C. Capponi, G. Cicoria, M. Parmeggiani, C. Salvarani, A. Versari, R. Corradini, M. Asti, ^{64}Cu and fluorescein labeled anti-miRNA peptide nucleic acids for the detection of miRNA expression in living cells. Sci Rep. 9 (2019) 3376. https://doi.org/10.1038/s41598-018-35800-x.

58. J. Gasparello, C. Papi, M. Zurlo, R. Corradini, R. Gambari, A. Finotti, Demonstrating specificity of bioactive peptide nucleic acids (PNAs) targeting microRNAs for practical laboratory classes of applied biochemistry and pharmacology. PLoS One. 14 (2019) e0221923. https://doi.org/10.1371/journal.pone.0221923.

59. E. Brognara, E. Fabbri, G. Montagner, J. Gasparello, A. Manicardi, R. Corradini, N. Bianchi, A. Finotti, G. Breveglieri, M. Borgatti, I. Lampronti, R. Milani, M.C. Dechecchi, G. Cabrini, R. Gambari, High levels of apoptosis are induced in human glioma cell lines by co-administration of peptide nucleic acids targeting miR-221 and miR-222. Int J Oncol. 48 (2016) 1029-1038. https://doi.org/10.3892/ijo.2015.3308.

60. V.V. Demidov, V.N. Potaman, M.D. Frank-Kamenetskii, M. Egholm, O. Buchard, S.H. Sönnichsen, P.E. Nielsen, Stability of peptide nucleic acids in human serum and cellular extracts. Biochem Pharmacol. 48 (1994) 1310-1313. https://doi.org/10.1016/0006-2952(94)90171-6.

61. M. Borgatti, A. Romanelli, M. Saviano, C. Pedone, I. Lampronti, L. Breda, C. Nastruzzi, N. Bianchi, C. Mischiati, R. Gambari R, Resistance of decoy PNA-DNA chimeras to enzymatic degradation in cellular extracts and serum. Oncol Res. 13 (2003) 279-287. https://doi.org/10.3727/096504003108748339.

62. U. Koppelhus, P.E. Nielsen, Cellular delivery of peptide nucleic acid (PNA).
Adv Drug Deliv Rev. 55 (2003) 267-280. https://doi.org/10.1016/s0169-409x(02)00182-5.

63. E. Rozners, Recent advances in chemical modification of Peptide nucleic acids. J Nucleic Acids. 2012 (2012) 518162. https://doi.org/10.1155/2012/518162.

64. M.E. Kreft, U.D. Jerman, E. Lasič E, N. Hevir-Kene, T. Lanišnik Rižner, L. Peternel, K. Kristan, The characterization of the human cell line Calu-3 under different culture conditions and its use as an optimized in vitro model to investigate bronchial epithelial function. Eur J Pharm Sci 69 (2015) 1-9. https://doi.org/ 10.1016/j.ejps.2014.12.017.

65. X. Xiang, X. Zhuang, S. Ju, J. Mu, L. Zhang, D. Miller, W. Grizzle, H.G. Zhang, miR-155 promotes macroscopic tumor formation yet inhibits tumor dissemination from

mammary fat pads to the lung by preventing EMT. Oncogene 30 (2011) 3440-3453. https://doi.org/ 10.1038/onc.2011.54.

66. T. Paul, S. Li, S. Khurana, N.S. Leleiko, M.J. Walsh, The epigenetic signature of CFTR expression is co-ordinated via chromatin acetylation through a complex intronic element. Biochem J. 408 (2007) 317-326. https://doi.org/10.1042/BJ20070282.

67. R. Gambari, J. Gasparello, E. Fabbri, M. Borgatti, A. Tamanini, A. Finotti, Peptide Nucleic Acids for MicroRNA Targeting. Methods Mol Biol. 2105 (2020) 199-215. https://doi.org/10.1007/978-1-0716-0243-0_12.

68. J. Gasparello, E. Fabbri, R. Gambari, A.Finotti, Differential effects on the miRNome of the treatment of human airway epithelial Calu-3 cells with peptide-nucleic acids (PNAs) targeting microRNAs miR-101-3p and miR-145-5p: next generation sequencing datasets. Data in Brief, *in the press.*

69. W. Wang, X. Xiao, X. Chen, Y. Huo, W.J. Xi, Z.F. Lin, D. Zhang, Y.F. Li, F. Yang, W.H. Wen, A.G. Yang, T. Wang, Tumor-suppressive miR-145 co-repressed by TCF4-β-catenin and PRC2 complexes forms double-negative regulation loops with its negative regulators in colorectal cancer, Int J Cancer 142 (2018) 308-321. https://doi.org/10.1002/ijc.31056.

70. M.C. Dechecchi, A. Tamanini, G. Cabrini. Molecular basis of cystic fibrosis: from bench to bedside, Ann Transl Med 6 (2018) 334-347. https://doi.org/10.21037/atm.2018.06.48.

71. M. Mijnders, B. Kleizen, I. Braakman I, Correcting CFTR folding defects by small-molecule correctors to cure cystic fibrosis. Curr Opin Pharmacol 34 (2017) 83-90. https://doi.org/10.1016/j.coph.2017.09.014.

71. M. Gentzsch, H.Y. Ren, S.A. Houck, N.L. Quinney, D.M. Cholon, P. Sopha, I.G. Chaudhry, J. Das, N.V. Dokholyan, S.H. Randell, D.M. Cyr. Restoration of R117H CFTR folding and function in human airway cells through combination treatment with VX-809 and VX-770. Am J Physiol Lung Cell Mol Physiol. 311 (2016) L550-L559. https://doi.org/ 10.1152/ajplung.00186.2016.

73. A.A. Aslam, C. Higgins, I.P. Sinha, K.W. Southern. Ataluren and similar compounds (specific therapies for premature termination codon class I mutations) for cystic fibrosis, Cochrane Database Syst Rev. 1 (2017) CD012040. https://doi.org/ 10.1002/14651858.CD012040.pub2.

74. A. Finotti, N. Bianchi, E. Fabbri, M. Borgatti, G. Breveglieri, J. Gasparello, R. Gambari, Erythroid induction of K562 cells treated with mithramycin is associated with inhibition of raptor gene transcription and mammalian target of rapamycin complex 1 (mTORC1) functions. Pharmacol Res. 91 (2015) 57-68. https://doi.org/ 10.1016/j.phrs.2014.11.005.

75. M. Favia, M.T. Mancini, V. Bezzerri, L. Guerra, O. Laselva, A.C. Abbattiscianni, L. Debellis, S.J. Reshkin, R. Gambari, G. Cabrini, V. Casavola, Trimethylangelicin promotes the functional rescue of mutant F508del CFTR protein in cystic fibrosis airway cells. Am J Physiol Lung Cell Mol Physiol. 307 (2014) L48-L61. https://doi.org/ 10.1152/ajplung.00305.2013.

76. F. Van Goor, S. Hadida, P.D. Grootenhuis, Burton B, J.H. Stack, K.S. Straley, C.J. Decker, M. Miller, J. McCartney, E.R. Olson, J.J. Wine, R.A. Frizzell, M. Ashlock, P.A. Negulescu, Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. Proc Natl Acad Sci USA 108 (2011) 18843-18848. https://doi.org/ 10.1073/pnas.1105787108.

FIGURES

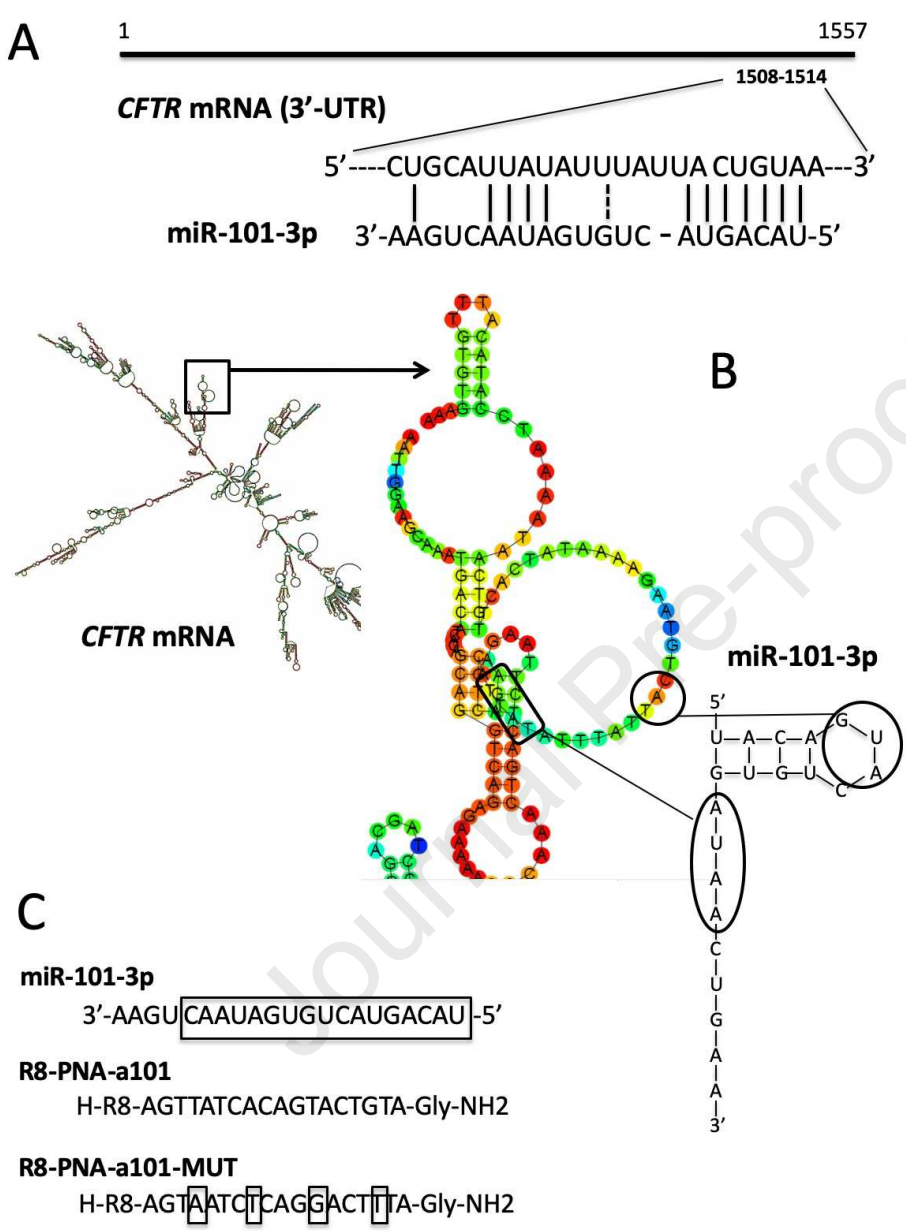

Figure 1. A. Computer-aided analysis of the possible pairing interaction between has-miR-101-3p (entry: http://www.mirbase.org/cgi-bin/mature.pl?mature_acc=MIMAT0000099) and *CFTR* mRNA (https://www.ncbi.nlm.nih.gov/nuccore/?term=CFTR). The MirWalk prediction database and the RNAfold WebServer (http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi) were used. The complementarity between miR-101-3p and the *CFTR* mRNA miR-101-3p binding site is shown. B. Magnification of the stem loop secondary structure possible interactions. C. Sequences of the R8-PNA-a101 and of the mutated R8-PNA-a101-MUT. The miR-101-3p binding sequence and the mutations in the R8-PNA-a101-MUT are boxed.

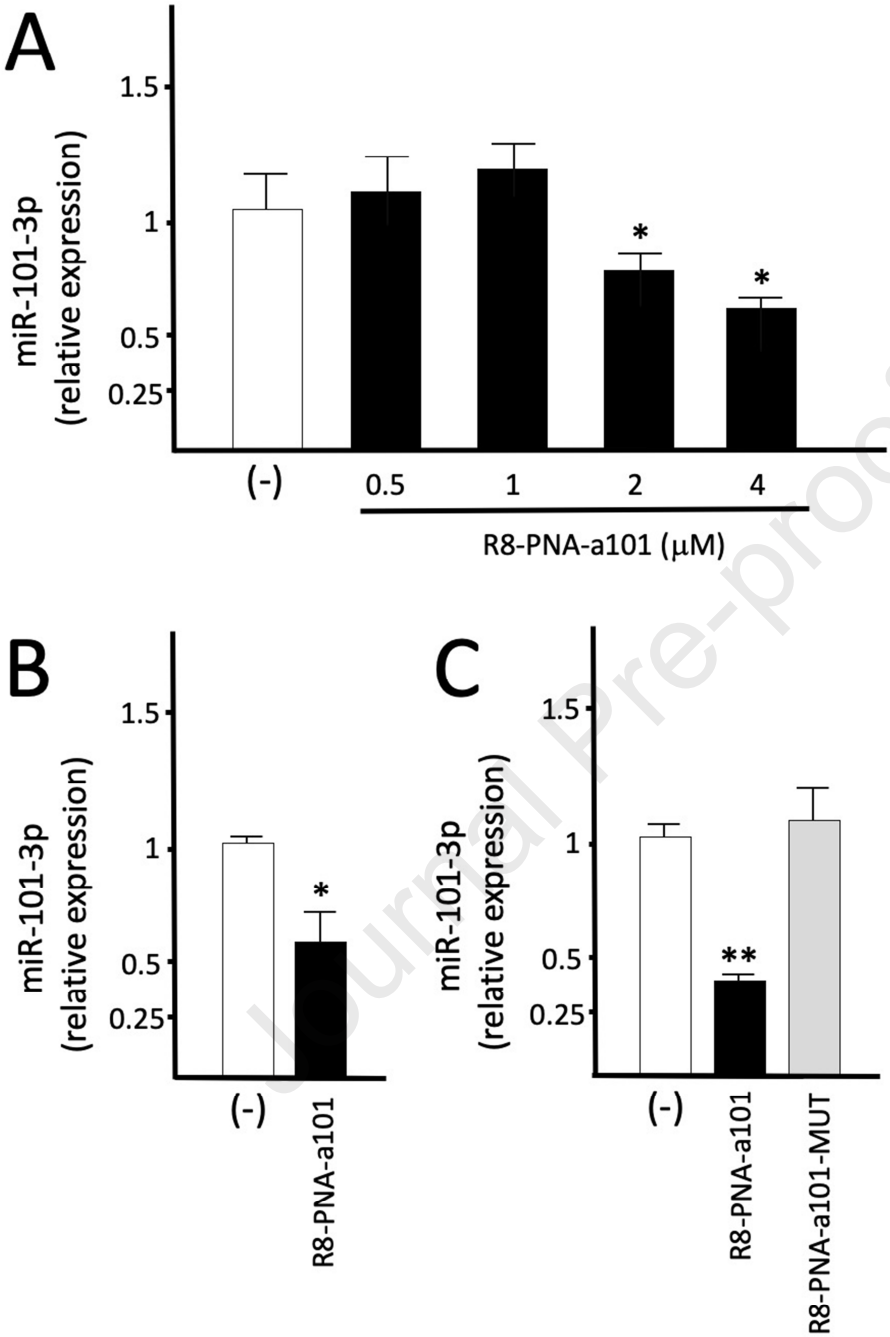

Figure 2. A. Effects on inhibition of miR-101-3p RT-qPCR signal in 72-hours treatment of Calu-3 cells with increasing amounts of R8-PNA-a101. B. Summary of the results obtained in independent experiments using 4 μM R8-PNA-a101. C. Inhibition of miR-101-3p in Calu-3 cells treated for 72 hours with R8-PNA-a101 (black box) and mutated R8-PNA-a101-MUT (gray box) molecules (4 μM). Results represent the average S.D. of three determinations (A), or five (B) and three (C) independent experiments.

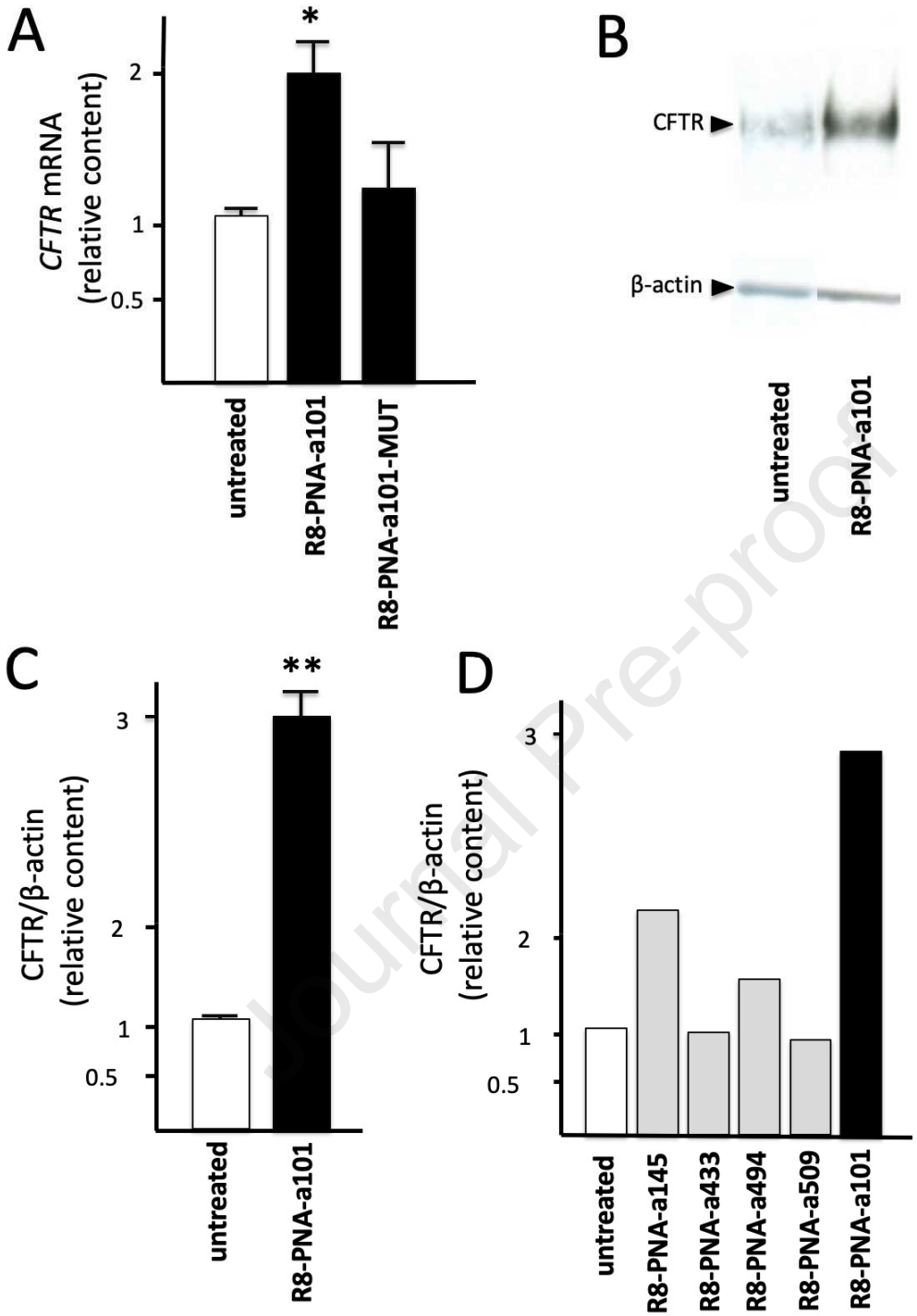

Figure 3. A-C. Upregulation of *CFTR* mRNA (RT-qPCR, panel B) and proteins (Western blotting of CFTR and β-actin, panels B and C) in Calu-3 cells cultured for 72 hours in the absence or in the presence of 4 μM R8-PNA-a101-3p, as indicated. The quantification of the CFTR/β-actin ratios is shown and the results are representative of three independent experiments (panel C). D. Effects of PNAs targeting different miRNAs demonstrated to bind the 3'-UTR sequence of the *CFTR* mRNA.

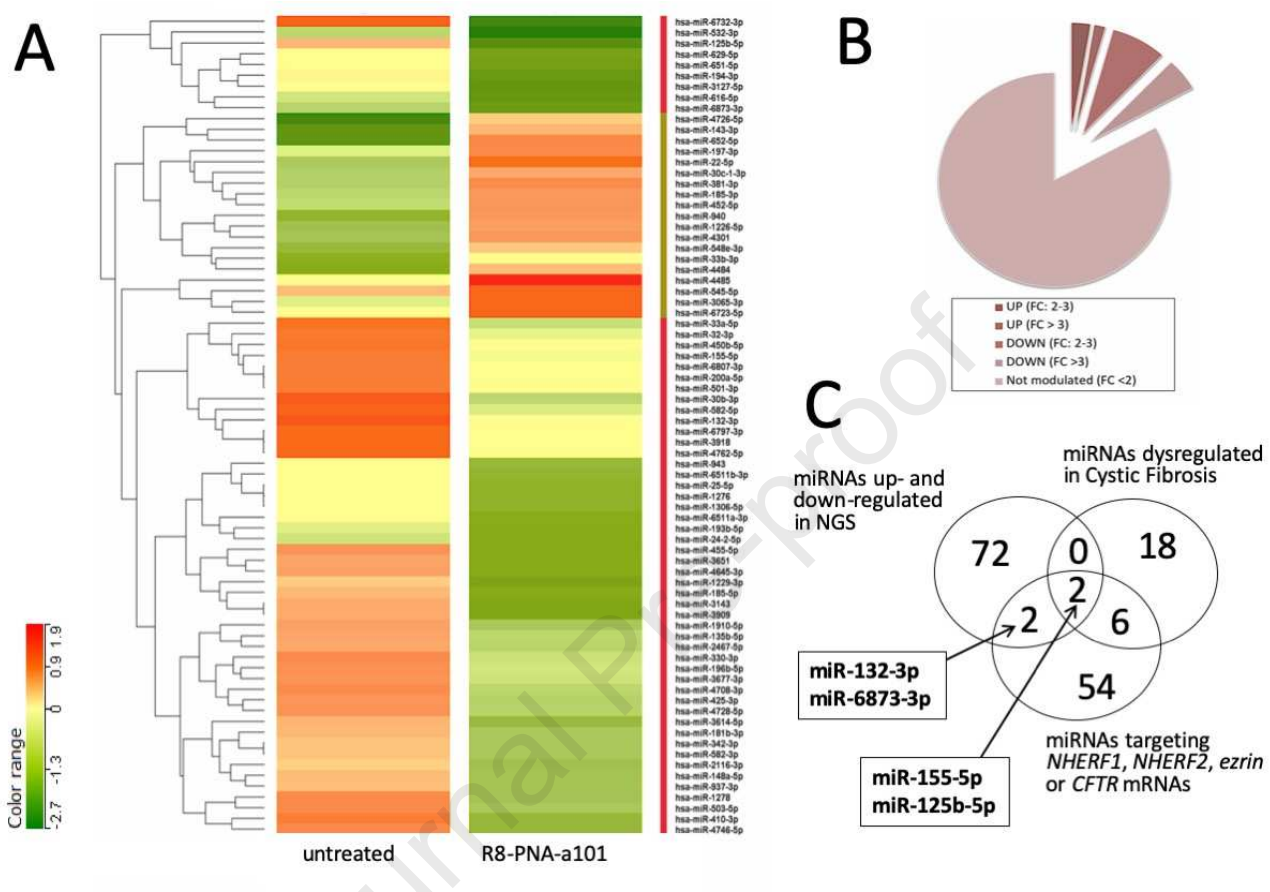

Figure 4. Effects of R8-PNA-a101 on miRNome profile analyzed by NGS sequencing. A. Calu-3 cells were cultured in the absence or in the presence of 4 μM R8-PNA-a101 for 48 hours, RNA was extracted and NGS performed as described in the Materials and Methods section. B. Summary of the number of miRNAs not modulated, up- and down-modulated. C. Venn diagram showing two miRNAs (one of which miR-155-5p) in common with the three lists (miRNAs up- and down-regulated in NGS, miRNAs dysregulated in CF and miRNA targeting *NHERF1*, *NHERF2*, *ezrin* or *CFTR* mRNAs).

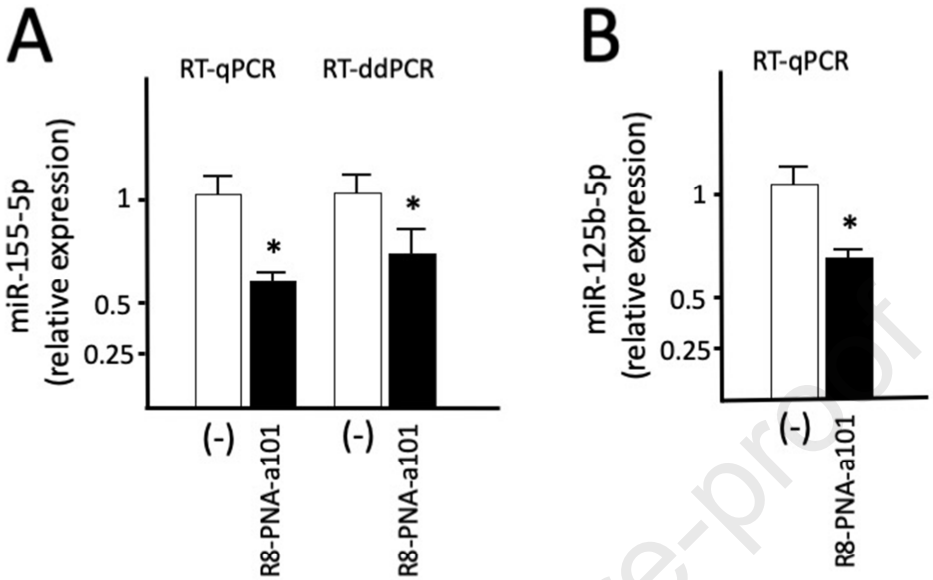

Figure 5. Validation of the R8-PNA-a101 mediated down regulation of miR-155-5p (A) and miR-125b-5p, analyzed by RT-qPCR. Data were derived by three independent experiments (average ± S.D.). The R8-PNA-a101 was used at 4 μM.

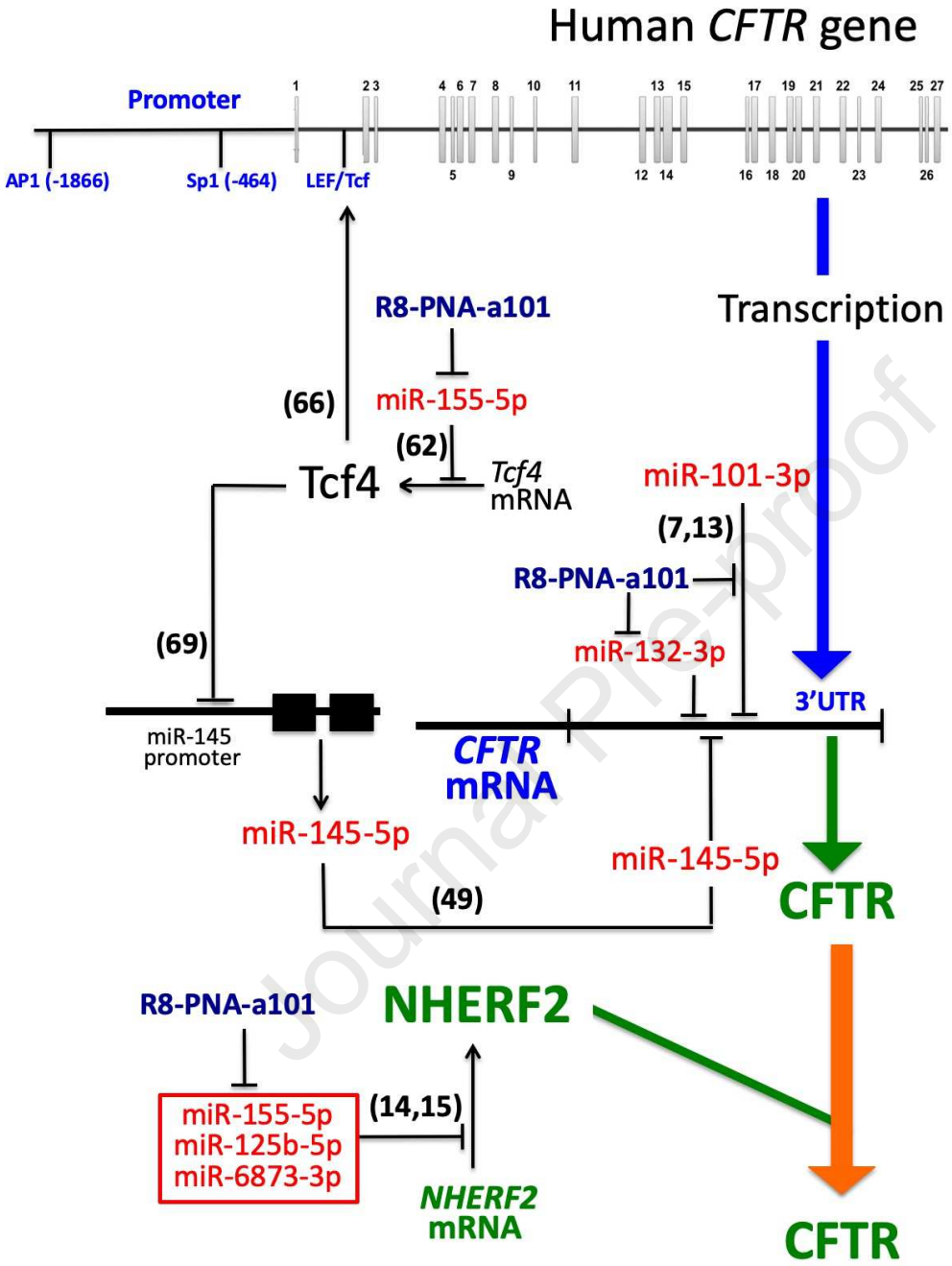

Figure 6. Proposed model for the interplay between transcription factors, CFTR regulation and miR-101-5p regulated molecules. The published information concerning specific steps are indicated in parenthesis. The effects of R8-PNA-a101 on miR-101-3p are presented in Figure 2. The effects of R8-PNA-a101 on miR-155-5p and miR-125b-5p are described in Figure 5. The effects of R8-PNA-a101 on CFTR are presented in Figure 3.

Table I. MicroRNAs targeting CFTR, NHERF1, NHERF2, Ezrin

miRNA	Target	miRNA	Target	miRNA	Target
let-7b-5p	NHERF1	miR-301b-5p	NHERF2	miR-5698	NHERF2
miR-101-3p	CFTR	miR-30a-5p	NHERF2	miR-593-3p	NHERF2
miR-1180-3p	Ezrin	miR-3120-5p	NHERF2	miR-600	CFTR
miR-1257	Ezrin	miR-328-3p	Ezrin	miR-607	CFTR
miR-125b-5p	NHERF2	miR-335-5p	CFTR, NHERF1	miR-615-3p	Ezrin
miR-132-3p	CFTR	miR-345-5p	NHERF2	miR-6719-3p	Ezrin
miR-1343-5p	NHERF2	miR-3622b-5p	Ezrin	miR-6730-3p	Ezrin
miR-144-3p	CFTR	miR-3660	Ezrin	miR-6812-3p	NHERF2
miR-145-5p	CFTR	miR-3677-5p	Ezrin	miR-6817-3p	NHERF2
miR-146a-3p	NHERF2	miR-3921	NHERF2	miR-6818-3p	NHERF2
miR-149-5p	Ezrin	miR-423-5p	NHERF1	miR-6818-5p	Ezrin
miR-155-5p	NHERF2	miR-4294	Ezrin	miR-6827-5p	NHERF2
miR-183-5p	Ezrin	miR-433-3p	CFTR	miR-6870-5p	NHERF2
miR-184	Ezrin	miR-4522	NHERF2	miR-6873-3p	NHERF2
miR-186-5p	Ezrin	miR-4526	Ezrin	miR-6888-3p	NHERF2
miR-18a-5p	Ezrin	miR-4653-5p	NHERF2	miR-6895-3p	NHERF2
miR-204-5p	Ezrin	miR-4711-3p	NHERF2	miR-7110-3p	NHERF2
miR-205-5p	Ezrin	miR-4723-5p	NHERF2	miR-7111-5p	NHERF2
miR-222-3p	Ezrin	miR-4766-5p	Ezrin	miR-877-3p	NHERF1
miR-223-3p	CFTR	miR-494-3p	CFTR	miR-92a-3p	Ezrin
miR-25-3p	Ezrin	miR-509-3p	CFTR	miR-939-5p	NHERF2
miR-301a-5p	NHERF2				

From: Chou et al. [14] and Huang et al. [15]. The involvement of NHERF1, NHERF2 and ezrin on CFTR expression can be found in Sharma et al., 2016 [16], Holcomb et al., 2014 [17] and Abbattiscianni, 2016 [18].

Table II. List of microRNAs dysregulated in Cystic Fibrosis

microRNA	Regulation	Experimental model system	References
Let-7e-5p	Up	DeltaF508-CFTR expressing cells	Endale Ahanda *et al.,* 2015 [20]
miR-101-3p	Up	bronchial brushing (CF vs non-CF)	Megiorni *et al.,* 2011 [13]
miR-125b-5p	Up	DeltaF508-CFTR expressing cells	Endale Ahanda *et al.,* 2015 [20]
miR-125b-5p	Up	DeltaF508-CFTR expressing cells	Endale Ahanda *et al.,* [20]
miR-126-3p	Down	bronchial brushing (CF vs non-CF)	Oglesby *et al.,* 2010 [21]
		airway epithelial cultures (CF vs non-CF)	Oglesby *et al.,* 2010 [21]
miR-1343	Down	16HBE14o-	Stolzenburg *et al.,* 2016 [22]
miR-138-5p	Up	airway epithelial cultures (CF vs non-CF)	Ramachandran *et al.,* 2012 [8]
miR-144-3p	Down	16HBE14o- cells	Hassan *et al.,* 2012 [7]
miR-145-5p	Up	bronchial brushing (CF vs non-CF)	Oglesby *et al.,* 2013 [10]
		CFB41o- vs 16HBE14o-	Oglesby *et al.,* 2013 [10]
		nasal cavity brushing (CF vs non-CF)	Megiorni *et al.,* 2013 [13]
miR-146a-5p	Down	bronchial brushing (CF vs non-CF)	Kiviall *et al.,* 2019 [23]
		CFB41o- vs 16HBE14o-	Montanini *et al.,* 2016 [24]
miR-155-5p	Up	CFB41o- vs 16HBE14o-	Montanini *et al.,* 2016 [24]
		IB3-1 CF vs IB3/S9	Bhattacharyya *et al.,* 2011 [25]
miR-16-5p	Down	IB3-1, F508del-CFTR HBE cells	Kumar *et al.,* 2015 [26]
miR-17-5p	Up	airway epithelial cultures (CF vs non-CF)	Oglesby *et al.,* 2015 [27]
miR-181b-5p	Up	CFB41o- vs 16HBE14o-	Pierdomenico *et al.,* 2017 [28]
miR-221-3p	Up	CFBE41o- versus 16HBE14o-	Oglesby *et al.,* 2015 [27]
miR-223-3p	Up	bronchial brushing (CF vs non-CF)	Oglesby *et al.,* 2013 [10]
		CFB41o- vs 16HBE14o-	Oglesby *et al.,* 2013 [10]
miR-224-5p	Up	oral buccal mucosal epithelial cells	Fan *et al.,* 2015 [29]
miR-31-5p	Down	CFB41o- vs 16HBE14o-	Weldon *et al.,* 2014 [30]
miR-370-3p	Up	CFB41o- vs 16HBE14o-	Montanini *et al.,* 2016 [24]
miR-384	Down	CFB41o- and 16HBE14o-	Viart *et al.,* 2015 [11]
miR-494-3p	Up	bronchial brushing (CF vs non-CF)	Megiorni *et al.,* 2011 [13]
		CFB41o- vs 16HBE14o-	Oglesby *et al.,* 2013 [10]
		bronchial brushing (CF vs non-CF)	Oglesby *et al.,* 2013 [10]
miR-509-3p	Up	airway epithelial cultures (CF vs non-CF)	Amato *et al.,* 2014 [31]
miR-708-5p	Up	CFB41o- vs 16HBE14o-	Montanini *et al.,* 2016 [24]
miR-93-5p	Down	IB3-1 cells (infected with Ps. Aeruginosa versus not infected)	Fabbri *et al.,* 2014 [32]
miR-9-5p	Down	CFB41o- vs 16HBE14o-	Sonneville *et al.,* 2017 [33]
miR-99b-5p	Up	DeltaF508-CFTR expressing cells	Endale Ahanda *et al.,* 2015 [20]

Table III. List of miRNAs dysregulated after R8-PNA-a101 treatment

microRNA	FC	Regulation	microRNA	FC	Regulation
miR-1226-5p	2.94	up	miR-381-3p	2.69	up
miR-1229-3p	3.65	down	miR-3909	4.20	down
miR-125b-5p	5.87	down	miR-3918	2.52	down
miR-1276	2.52	down	miR-410-3p	4.59	down
miR-1278	3.36	down	miR-425-3p	2.50	down
miR-1306-5p	2.52	down	miR-4301	2.94	up
miR-132-3p	3.06	down	miR-4484	3.43	up
miR-135b-5p	2.04	down	miR-4485	5.20	up
miR-143-3p	5.39	up	miR-450b-5p	2.17	down
miR-148a-5p	2.32	down	miR-452-5p	2.20	up
miR-155-5p	2.14	down	miR-455-5p	4.76	down
miR-181b-3p	2.19	down	miR-4645-3p	4.20	down
miR-185-3p	2.26	up	miR-4708-3p	2.52	down
miR-185-5p	3.57	down	miR-4726-5p	6.37	up
miR-1910-5p	2.55	down	miR-4728-5p	2.48	down
miR-193b-5p	2.52	down	miR-4746-5p	4.08	down
miR-194-3p	4.08	down	miR-4762-5p	2.52	down
miR-196b-5p	2.04	down	miR-501-3p	2.04	down
miR-197-3p	2.00	up	miR-503-5p	3.06	down
miR-200a-5p	2.04	down	miR-532-3p	5.03	down
miR-2116-3p	2.16	down	miR-545-5p	2.12	up
miR-22-5p	3.92	up	miR-548e-3p	2.55	up
miR-24-2-5p	2.30	down	miR-582-3p	2.04	down
miR-2467-5p	2.04	down	miR-582-5p	3.06	down
miR-25-5p	2.52	down	miR-616-5p	3.36	down
miR-3065-3p	2.94	up	miR-629-5p	3.36	down
miR-30b-3p	4.20	down	miR-651-5p	3.57	down
miR-30c-1-3p	2.17	up	miR-6511a-3p	2.81	down
miR-3127-5p	4.20	down	miR-6511b-3p	2.38	down
miR-3143	4.20	down	miR-652-5p	7.84	up
miR-32-3p	2.30	down	miR-6723-5p	2.65	up
miR-330-3p	2.27	down	miR-6732-3p	15.10	down
miR-33a-5p	2.92	down	miR-6797-3p	2.52	down
miR-33b-3p	2.61	up	miR-6807-3p	2.04	down
miR-342-3p	2.04	down	miR-6873-3p	2.52	down
miR-3614-5p	2.81	down	miR-937-3p	2.24	down
miR-3651	4.08	down	miR-940	3.92	up
miR-3677-3p	2.04	down	miR-943	2.25	down

Treatment of human airway epithelial Calu-3 cells with a Peptide-Nucleic Acid (PNA) targeting the microRNA miR-101-3p is associated with increased expression of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene

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HIGHLIGHTS

- microRNAs are deeply involved in the regulation of Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene
- miR-101-3p targets the 3'-UTR sequence of the *CFTR* mRNA
- In the human airway epithelial Calu-3 cells miR-101-3p can be selectively inhibited by a peptide nucleic acid (PNA) carrying a full complementary sequence
- PNA-mediated inhibition of miR-101-3p was accompanied by CFTR up-regulation
- Next Generation Sequencing (NGS) was performed in order to verify the effects of the anti-miR-101-3p PNA on the Calu-3 miRNome, showing specific effects accompanied by alteration of other miRNAs, some of them involved in Cystic Fibrosis (CF)

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: