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Targeting microRNAs involved in human diseases: A novel approach for modification of gene expression and drug development

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ABSTRACT

The identification of all epigenetic modifications (i.e. DNA methylation, histone modifications and expression of noncoding RNAs such as microRNAs) involved in gene regulation is one of the major steps forward for understanding human biology in both normal and pathological conditions and for development of novel drugs. In this context, microRNAs play a pivotal role. This review article focuses on the involvement of microRNAs in the regulation of gene expression, on the possible role of microRNAs in the onset and development of human pathologies, and on the pharmacological alteration of the biological activity of microRNAs. RNA and DNA analogs, which can selectively target microRNAs using Watson–Crick base pairing schemes, provide a rational and efficient way to modulate gene expression. These compounds, termed antago-miR or anti-miR have been described in many examples in the recent literature and have proved to be able to perform regulatory as well as therapeutic functions. Among these, a still not fully exploited class is that of peptide nucleic acids (PNAs), promising tools for the inhibition of miRNA activity, with important applications in gene therapy and in drug development. PNAs targeting miR-122, miR-155 and miR-210 have already been developed and their biological effects studied both *in vitro* and *in vivo*.

1. Introduction

The identification of all epigenetic modifications involved in gene expression is one of the major steps forward for understanding human biology in both normal and pathological conditions. This field is referred to as epigenomics, and it is defined as epigenetic changes (i.e. DNA methylation, histone modification and expression of noncoding RNAs such as microRNAs) on a genomic scale [1]. In this context, microRNAs play a pivotal role.

MicroRNAs (miRNAs, miRs) are a family of small (19–25 nucleotides in length) noncoding RNAs that regulate gene expression by sequence-selective targeting of mRNAs, leading to a translational repression or mRNA degradation, depending on the degree of complementarity between miRNAs and the target mRNA sequences [2–5]. Since their discovery and first characterization, the number of microRNA sequences deposited in the miRBase databases is growing [6–10]. Considering that a single miRNA can target several mRNAs and a single mRNA might contain in the 3′UTR sequence several signals for miRNA recognition, it is calculated that at least 10–40% of human mRNAs are a target for microRNAs [10–13]. Hence, great interest is concentrated on the identification of validated targets of microRNAs.

This specific field of microRNA research has confirmed that the complex networks constituted by miRNAs and RNA targets coding for structural and regulatory proteins lead to the control of highly regulated biological functions, such as differentiation, cell cycle and apoptosis [1–3]. Low expression of a given miRNA is expected to be linked with a potential expression of targets mRNAs. Conversely, high expression of miRNAs is expected to induce low expression of biological functions of the target mRNAs [1–3].

Alteration of microRNA expression has been demonstrated to be associated with human pathologies as well as guided alterations of miRNAs have been suggested as a novel approach to develop innovative therapeutic protocols. MicroRNA therapeutics appears as a novel field in which miRNA activity is the major target of the

Abbreviations: miRNA (miR), microRNA; pri-miRNA, primary miRNA; pre-miRNA, precursor miRNA; RISC, RNA-induced silencing complex; ODN, oligodeoxyribonucleotide; PNA, peptide nucleic acid; LNA, locked nucleic acid; RNA Pol II, RNA polymerase II; TF, transcription factor; mitron, intron containing miRNA sequences; hESC, human embryonic stem cells; EB, embryoid body; UCB, umbilical cord blood; HbF, fetal hemoglobin; ErPCs, erythroid precursor cells; MTH, mithramycin; HPFH, high persistence of fetal hemoglobin; EPO, erythropoietin.

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intervention [14–17]. MiRNA inhibition can be readily achieved by the use of small miR-inhibitor oligomers, including RNA, DNA, DNA analogs (miRNA anti-sense therapy) [14,15]. On the contrary, increase of miRNA function (miRNA replacement therapy) can be achieved by the use of modified, suitably delivered miRNAs mimetics, transfection using recombinant vectors or lentivirus carrying miRNA gene sequences [16,17].

This review article focuses on the involvement of microRNAs in the regulation of gene expression, on the possible role of microRNAs in the onset and development of human pathologies, and on the pharmacological alteration of the biological activity of microRNAs using anti-miR molecules.

2. Biogenesis of microRNAs and drug design

Some miRNAs are encoded by unique genes (intergenic miRNAs) [18–23] and others are embedded into the intronic regions of protein-coding genes (intragenic miRNAs) [24–28]. Examples of intergenic miRNA are miR-210, miR-10a, miR-21, and miR-222/miR-221, which are encoded by unique genes located in the chromosome 11, 17, 17, 6 and X, respectively. The transcription is controlled, as protein-coding genes, by a promoter which is regulated by specific interactions with transcription factors (Fig. 1A). The transcription by RNA polymerase II of these miR genes gives rise to long primary miRNAs (pri-miRNAs) with typical stem-loop structures. These are rapidly processed by the nuclear RNase endonuclease-III Drosha, which, removing the branches, gives rise to precursor miRNAs (pre-miRNA) of around 60–100 nts in length (Fig. 1A).

An example of intragenic miRNA is miR-301. Its genomic sequences are embedded into the intronic regions of ska2 [27]. In this specific case, the transcription of miRNA sequences depends on the cellular promoter of the host gene. The miR sequences follow the splicing pathways giving rise to a "Mirtron" (microRNA/intron) sequence further processed by debranch enzymes to generate a pre-miRNA (Fig. 1B). The microRNA transcription can be controlled by targeting regulatory transcription factors, the microRNA promoter itself, or the promoter of the host gene. An example is that reported by Xi et al., showing that knocking-down of C-EBP-β induces a decrease of the recruitment of this transcription factor on the promoter of the LOC554202 gene (hosting miR-31) and down-regulation of miR-31 [28]. Another

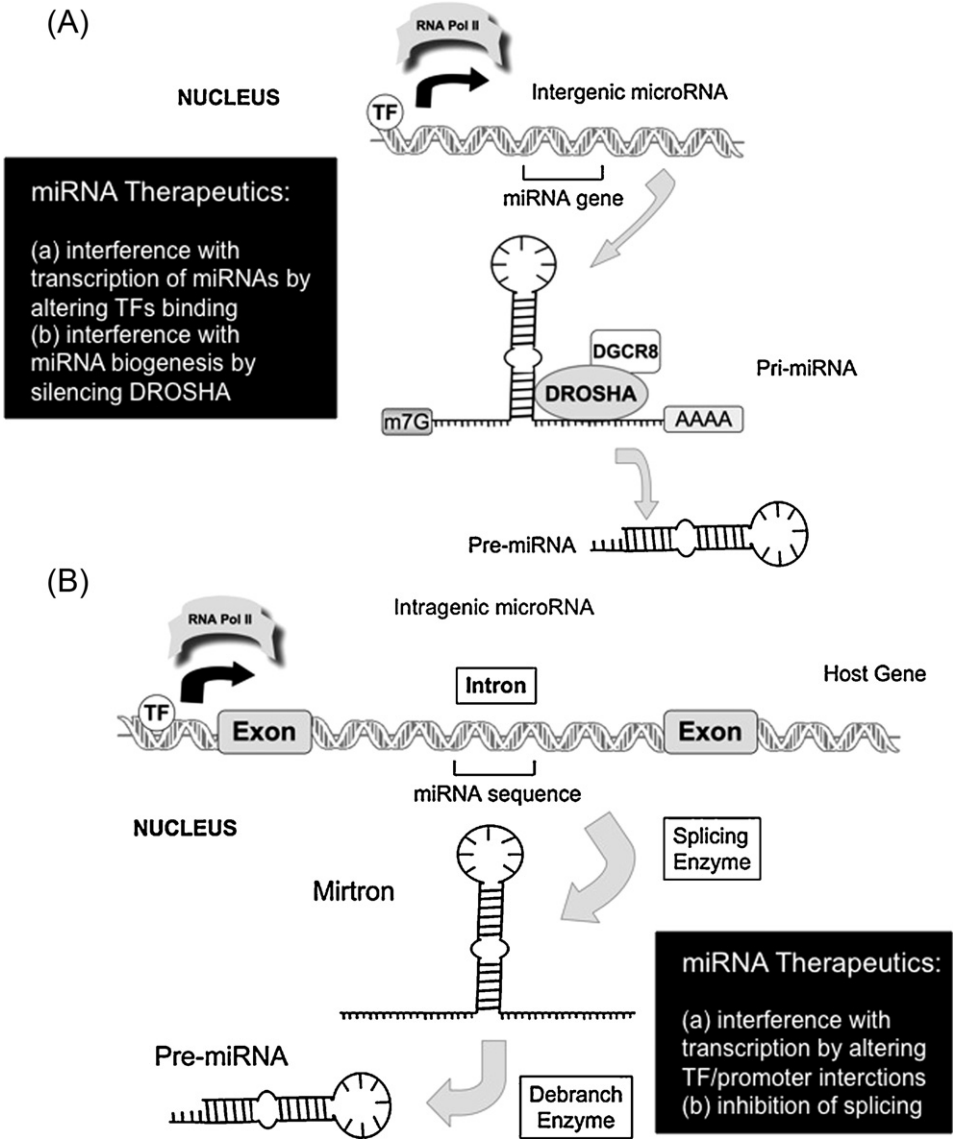

Fig. 1. Biogenesis of miRNAs (I) and possible pharmacological interventions to alter the generation of mature miRNAs. (A) Synthesis of pre-miRNAs by intergenic microRNAs. (B) Synthesis of pre-miRNAs by intragenic microRNAs. For further details on microRNA interference, several reviews are available [60–63].

example is the knockdown of the transcription factor Foxo3a, resulting in an increase in miR-21 levels, in agreement with the function of this transcription factor, which negatively regulates miR-21 transcription [22].

In both cases of intergenic miRNAs and intragenic miRNAs, the pre-miRNAs are transported from the nucleus to the cytoplasm by exportin-5. In the cytoplasm, pre-miRNAs are further processed by another RNase endonuclease-III (Dicer) to generate mature miRNAs around 22-nt long, which generate the RNA-induced silencing complex RISC (Fig. 2). The most widely used approach to alter processing or targeting functions of microRNA is the anti-sense strategy, which have been reported in several papers in which microRNAs have been targeted by anti-miR molecules [7–9]. This antisense strategy appears to be more specific in respect to targeting of transcription factors or miRNA promoters, since it affects single microRNAs or microRNA families, while targeting transcription factors or transcription factors binding sites is expected to have deep effects on the whole transcriptome, due to the fact that a single transcription factor is able to bind and regulate several genes.

In addition, recent reports suggest that miRNAs can be packaged in exosome fractions, followed by release of exosome/miRNAs from producing cells into body fluids [29–31]. This last feature has important diagnostic/prognostic implications [31].

3. Involvement of microRNAs in the control of gene expression

The basic mechanism leading to alteration of gene expression is based on the recruitment of mature miRNA at the level of the RISC silencing complex [32–37]. This process occurs in the cytoplasm, where the pre-miRNA hairpin is cleaved by the RNase III enzyme Dicer, which interacts with the 3′ end of the hairpin and cuts away the loop joining the 3′ and 5′ arms, yielding an imperfect miRNA/miRNA duplex. One of the strands is incorporated into the RISC, where it binds to target mRNA sequences. Animal miRNAs are usually complementary to a site in the 3′UTR. Perfect or near perfect base pairing with the target RNA promotes cleavage of the RNA [35–37]. It is proposed that in the case of partially complementary microRNAs, in order to recognize their targets, nucleotides 2–7 of the miRNA (the 'seed region') are important [32–34]. This is the key

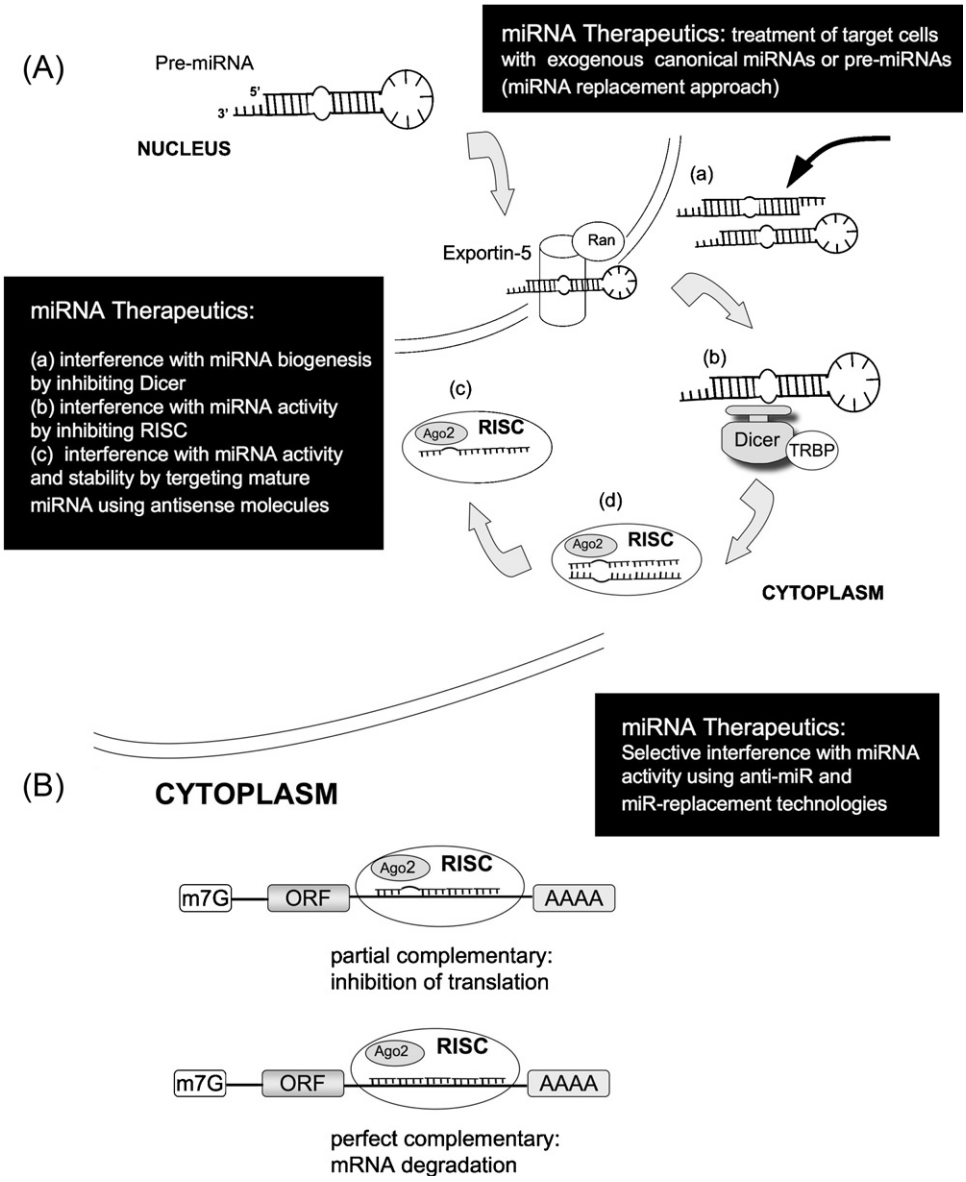

Fig. 2. Biogenesis of miRNAs (II) and possible pharmacological interventions to alter the generation of mature miRNAs. (A) Generation of the RISC. (B) Interactions with the target mRNA sequences [60–63].

process permitting mature miRNAs to exert their effects in gene regulation. The final effect of miRNAs activity is the inhibition of the synthesis of the protein(s) encoded by the target mRNA(s).

This has of course important biological implications depending on the role of the protein in the cellular network. Since a single 3′UTR of a given mRNA contains signal sequences for several microRNAs, applied biological studies are needed to determine which microRNA should be targeted to achieve alteration of gene expression. Possible effects on the expression of other mRNA targets should be considered. An alteration of a single microRNA may exhibit multiple effects, possibly in combination with the targeting activity of other miRNAs, enabling the achievement of strong biological effect [14–17].

4. Involvement of microRNAs with specialized functions: miRNAs, erythroid differentiation and γ-globin gene expression

4.1. MicroRNAs in erythropoiesis

Increasing numbers of published studies report the involvement of microRNAs in erythropoiesis [13,38–41]. Different cellular experimental systems were used in these studies. Huang et al. employed human embryonic stem cells (hESCs) as a model system to study early human hematopoiesis [42]. These authors differentiated hESCs by embryoid body (EB) formation and compared the miR expression profile of undifferentiated hESCs to CD34(+) EB cells, demonstrating the function of miRs-126/126* in the negative regulation of erythropoiesis [42]. Felli et al. identified miR-221 and miR-222 as being highly expressed in the human cord blood derived hematopoietic $CD34^+$ progenitor cells [38]. MicroRNA expression profiling was also performed by Choong et al. on *ex vivo* differentiating erythroid cultures derived from human umbilical cord blood (UCB) CD34 cells and K562 cells to identify miRNAs involved in erythropoiesis [39]. After comparison of stimulated UCB-derived $CD34^+$ cells and K562 cells, several miRNAs were identified as putatively critical for erythroid development and maturation. MicroRNAs miR-15b, miR-16, miR-22, and miR-185 were found to have strong positive correlation with the appearance of erythroid surface antigens (CD71, CD36, and CD235a) and hemoglobin synthesis, while miR-28 displayed an inverse relationship with the expression of these markers. Other efforts aimed at defining erythroid-specific miRNAs were those published by Georgantas et al., who demonstrated that miR-155 is involved in the control of both myeloid and erythroid differentiation [40]. In conclusion, miRNAs have been shown to play a role in normal hematopoiesis [13,36–40]. Zebrafish embryos are another model system which has been demonstrated to be important in defining the role of microRNA in hematopoiesis. Using this system, Grabher et al. demonstrated that miR-126 is a novel physiological regulator of proto-oncogene c-myb during definitive hematopoiesis; knockdown of miR-126 leads to increased c-Myb levels and promotes erythropoiesis *in vivo* [43].

4.2. miR-210 and erythroid differentiation

The miRNA-profile in erythroid precursor cells from normal and thalassemic patients expressing different levels of fetal

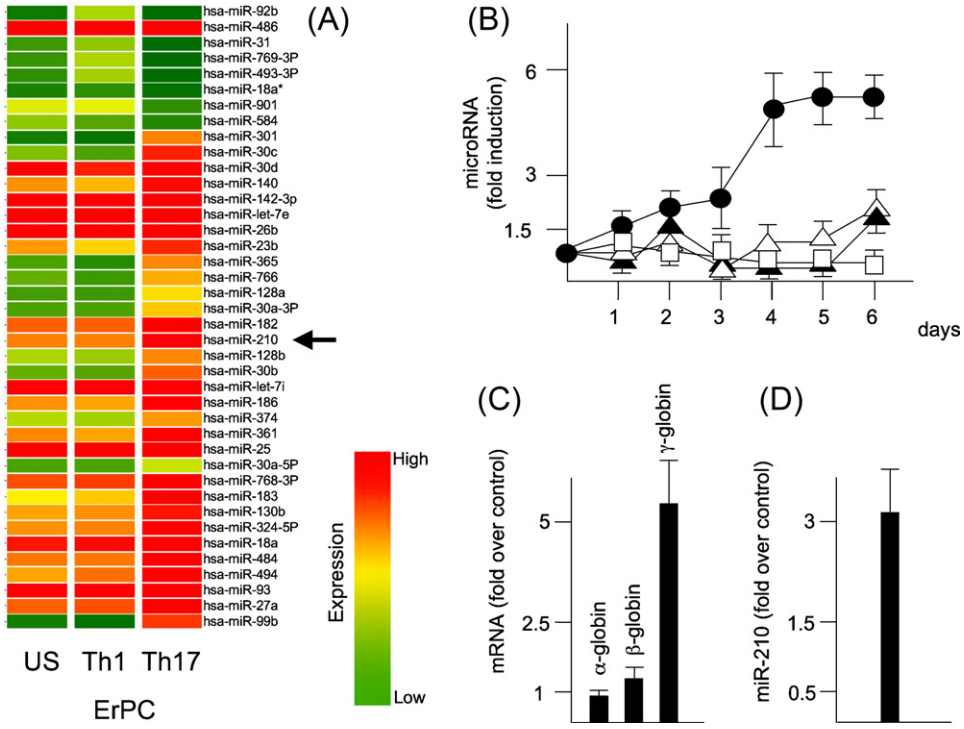

Fig. 3. (A) Profiles of miRNA expression in erythroid cell cultures. The analysis was performed with total RNA from US (unaffected subject), Th1 (a thalassemic patient producing low levels of HbF) and Th17 (a thalassemic patient producing high levels of HbF) samples, as indicated. RNA analysis using microRNA microarray chips has been carried on by Agilent Technologies [13] using a platform containing 470 human miRNA probes for mature and precursor of microRNAs. RNA was isolated from erythroid precursor cells (ErPCs) following the method developed by Eitan Fibach and elsewhere described [40]. The Heatmap of miRNA expression profiling is depicted using a color-bar approach. Raw data were normalized and analyzed by GeneSpring GX software version 7.3 (Agilent Technologies). (B) Kinetics of increase of miR-210 (●), miR-155 (△), miR-221 (▲) and miR-222 (□) sequences following treatment of human leukemic K562 cells with 30 nM mithramycin (MTH). For microRNA quantification reverse transcriptase (RT) reactions were performed using TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) and real-time PCR were performed using the specific human TaqMan MicroRNA Assay Kits from Applied Biosystems for the different miRNA sequences. Data represent fold increase in respect to control untreated cells (average ± S.D. from three determinations). (C, D) Fold induction of globin mRNAs (C) and miR-210 (D) in K562 cells induced to erythroid differentiation by 30 nM MTH.
Modified from Bianchi et al. [13].

hemoglobin (HbF) (some of them displaying hereditary persistence of fetal hemoglobin, HPFH, phenotype) was analyzed in our laboratory by microarrays [13]. Two approaches were followed for microRNA expression studies, including (a) miRNA profiling and (b) quantitative RT-PCR. RNA was isolated from unaffected subjects, β-thalassemia patients expressing low HbF levels and β-thalassemia-HPFH patients, labeled and hybridized on microRNA microarray chips [13]. The microarray data (see Fig. 3A) allowed us to identify miR-210 as the highly expressed miRNA in the erythroid precursor cells (ErPCs) from a HPFH patient among those displaying similar expression in ErPCs from unaffected subjects and β-thalassemia patients expressing low HbF levels [13]. When RT-PCR was performed on MTH-induced K562 cells and erythroid precursor cells [44], we demonstrated that miR-210 is induced in time-dependent and dose-dependent fashion (Fig. 3B–D). In erythroid precursor cells from human donors miR-210 is also induced following MTH-treatment. On the basis of these data, miR-210 is proposed as associated with erythroid differentiation and induction of HbF synthesis. MiR-210 has been recently associated with hypoxia [45–47], as demonstrated by Kulshreshtha et al., who described the microRNA signature of hypoxia, which includes high expression of miR-210 [48]. Interestingly, hypoxia has been recently demonstrated to alter progression of the erythroid program [49–51]. Low pO_2 may indeed modulate the relative amounts and types of hemoglobins produced by erythroid cells, leading to increased HbF during stress erythropoiesis [51].

The involvement of miR-210 in erythroid differentiation was also reported by Kosaka et al. using UT-7 cells as a model system [52]. Human miRNA microarrays were used to analyze miRNA expression in the erythropoietin (EPO)-dependent cell line UT-7/EPO. Among 324 human miRNAs, miR-188, miR-362 and miR-210 levels were significantly high in UT-7/EPO cells, and stimulation with EPO in UT-7 cells increased the level of these three miRNAs. Knockdown of miR-210 in UT-7/EPO cells led to apoptosis. In mouse fetal liver cells, the expression of miR-210 was high during erythroid maturation *in vitro*. Together, these data suggest miR-210 to be a member of a new class of regulatory miRNAs that might play an important role in erythroid maturation [52].

4.3. MicroRNAs and expression of γ-globin genes

The developmental progression of globin gene expression and the reactivation of γ-globin gene expression associated with HbF synthesis in the adult is an important therapeutic strategy for sickle cell anemia and β-thalassemia [53]. Patients with HPFH exhibit a positive clinical status, since the activation of γ-globin genes and the synthesis of HbF partly overcomes the problems caused by the lack of HbA in thalassemia syndromes and the excess of free α-globins in red-blood cells [53,54]. Sankaran et al. reported that increased expression of miR-15a and miR-16-1 in primary human erythroid progenitor cells results in high fetal and embryonic hemoglobin gene expression, of interest when considered together with the finding that the direct target of these microRNAs is MYB, which plays an important role in silencing the fetal and embryonic hemoglobin genes [41]. This finding has relevance for the therapy of β-thalassemia. Other miRNAs implicated in HbF induction are miR-221/222, whose expression increases during post-natal development. In functional studies, overexpression of these miRs in cord blood progenitors caused a remarkable decrease in Kit expression, erythroblast proliferation and HbF content, whereas their suppression induced opposite effects [55]. This study was the basis of the recent patent US20090215862 (microRNA, http://www.freepatentsonline.com/) outlining a possible employment of microRNA therapeutics based on miR-221, miR-222, miR-130a and miR-130b for treatment of kit-dependent tumors (mainly gastro-intestinal stromal tumor, acute leukemias, erythroleukemia, papillary thyroid carcinoma) or hematological diseases, wherein the therapy is the modulation of erythropoiesis.

5. MicroRNAs and human pathologies

Strong evidences are reported by a number of authors suggesting the concept that the inappropriate expression of miRNA is associated with cancer [56–61] and a variety of other pathologies [62–71]. For example, *let*-7 miRNA prevents proliferation of cancer stem cells [72]. miRNAs have roles in obesity [73,74] and diabetes [75], hearing loss in humans [76], development of liver diseases [77], osteopenic diseases [63], kidney diseases [64], schizophrenia [78–80], Tourette's syndrome [78], psoriasis [81,82], Fragile-X mental retardation syndrome [83], polycythemia vera [84,85], AIDS [86,87], cardiovascular, muscular and neurodegenerative diseases [68]. Thus, molecules that alter the function or abundance of specific miRNAs represent a new strategy for treating human diseases.

These studies have produced a large number of miRNA-disease associations and show that the mechanism of action of miRNAs implicated in disease is very complex. A large-scale analysis and integration of these miRNA-disease associations at a system level offers a platform to dissect the mechanisms of miRNAs in disease, although the current miRNA-disease associations are far from complete. In the Human MicroRNA Disease Database (HMDD, http://202.38.126.151/hmdd/mirna/md/), 270 diseases, 1243 publications, and 3017 miRNA-disease associations are reported [88,89]. Table 1 reports selected examples of miRNAs involved in human diseases, also indicating target mRNAs and biological effects [90–101].

6. MicroRNA and cancer

MicroRNAs play a pivotal role in cancer [102–108]. The literature on this specific issue is impressive (see the Human MicroRNA Disease Database, http://202.38.126.151/hmdd/mirna/md/) [61,62,102–142]. MicroRNAs play a double role in cancer, behaving both as oncogenes [141,142] or tumor suppressor genes [133–137]. In general, miRNAs promoting cancer target mRNA coding for tumor-suppression proteins, while microRNAs exhibiting tumor-suppression properties usually target mRNAs coding oncoproteins. MicroRNAs which have been demonstrated to play a crucial role in the initiation and progression of human cancer are defined as oncogenic miRNAs (oncomiRs) [57,141,142]. Moreover, microRNAs have been definitely demonstrated to be involved in cancer metastasis (metastamiRs) [61,62]. Tables 2 and 3 show representative examples of oncomiRs (Table 2) and onco-suppressor miRNAs (Table 3).

For instance, miR-372 and miR-373 were identified as oncogenes, after the screening of hundreds of miRNAs [138]. The mechanism of action of these microRNAs is to negatively regulate the expression of the LAST2 tumor suppressor gene, thus blocking the pathway of one of the key tumor suppressors, p53 [138]. Using breast cancer MCF7 as a model system, Huang et al. were able to demonstrate that miR-373 promotes tumor invasion and metastasis [139]. A similar tumor-promoting activity is exhibited by miR-221 and miR-222, able to stimulate proliferation following inhibition of the expression of the tumor suppressor p27Kip1 [138].

An opposite effect on tumor development is displayed by other miRNAs; for instance miR-31 expression levels correlate inversely with the metastatic ability of breast tumor cell lines, and the inhibition of miR-31 promotes metastasis [140]. Further studies have revealed that miR-31 blocks several steps of metastasis,

Table 1
MiRNAs and experimental data supporting an involvement in pathologies.

MicroRNA	Disease	Biological effects	Target mRNA/pathway	Reference
miR-143	Obesity-associated diabetes	Inhibition of insulin-stimulated AKT activation and glucose metabolism: development of obesity-induced insulin resistance	ORP 8	[90]
miR-519d	Obesity	Increased lipid accumulation during preadipocyte differentiation: metabolic imbalance and subsequent adipocyte hypertrophy in subcutaneous adipose tissue (SAT)	PPARA	[91]
miR-375	Diabetes	Enhance islet function and combat β-cell failure: regulator of glucose-stimulated insulin gene expression and proliferation of pancreatic β-cells	PDK1	[92]
miR-122	HCV infection	Antiviral targets regulating HCV gene expression; liver-specific	5′ NCRs of HCV	[93]
miR-29	Osteopenic disease	Decreased osteonectin protein during the matrix maturation and mineralization phases of late differentiation	Negative regulators of Wnt signaling, Dkk1, Kremen2 and sFRP2	[94]
miR-203	Psoriasis	Dysfunction of the cross talk between resident and infiltrating cells	SOCS-3	[95]
miR-18; miR-19	Age-related heart failure	Down-regulation of CTGF and TSP-1	CTGF and TSP-1	[96]
miR-21	Myocardial disease	Stimulating MAP kinase signaling	SPRY1	[97]
miR-107	Alzheimer's disease	Acceleration of the disease progression	BACE1	[98]
miR-143; miR-145	Vascular diseases atherosclerosis (ES mouse)	Promotion of the differentiation and repression of proliferation of smooth muscle cells	Targeting a network of transcription factors: Myocd, Nkx2-5 including Klf4, Elk-1	[99]
miR-96	Nonsyndromic progressive hearing loss	Mutation in miR leading loss of function	Aqp5; Celsr2; Myrip; Odf2; Ryk	[100,101]

Abbreviations: ORP 8, oxysterol-binding-protein-related protein; PPARA, peroxisome proliferator-activated receptor-α; PDK1, 3′-phosphoinositide-dependent protein kinase-1; NCRs, non-coding regions; HCV, hepatitis C virus; Dkk1, Dikkopf-1; sFRP2, secreted frizzled related protein 2; SOCS-3, suppressor of cytokine signaling 3; CTGF, connective tissue growth factor; TSP-1, thrombospondin 1; SPRY1, protein sprouty homolog 1; BACE1, β-site amyloid precursor protein-cleaving enzyme 1; Myocd, myocardin; Nkx2-5, NK2 transcription factor related, locus 5; Klf4, Kruppel-like factor 4; Elk-1, E twenty-six (ETS)-like transcription factor 1; Aqp5, aquaporin 5; Celsr2, cadherin, EGF LAG seven-pass G-type receptor 2 (flamingo homolog, Drosophila); Myrip, myosin VIIA and Rab interacting protein; Odf2, outer dense fiber of sperm tails 2; and Ryk, RYK receptor-like tyrosine kinase.

Table 2
MiRNAs exhibiting oncogene function in cancer (oncomiRs).

MicroRNA	Disease	Biological effects	Target mRNA/pathway	Reference
miR-182	Melanoma	Promotion of melanoma metastasis	MITF and FOXO3	[102]
miR-221; miR-222	Atypical teratoid/rhabdoid tumors (ATRT)	Inhibition of the tumor suppressor and inhibitor of cell cycle $p27^{Kip1}$	$p27^{Kip1}$	[103]
miR-221; miR-222	Glioblastoma	Inhibition of cell apoptosis	PUMA	[104]
miR-21	Breast, colon, pancreas, lung, prostate, liver, and stomach cancer; AML(11q23); chronic lymphocytic leukemia; glioblastoma	Stimulation of cellular proliferation, apoptosis, and migration; action on mitochondrial apoptosis tumor-suppressive pathways	PTEN, PDCD4, TPM1, P53 and TGF-β	[105–110]
miR-122a	Hepatocellular carcinoma	Cell cycle regulation, DNA repair, carcinogenetic process leading to HCC development	CCNG1	[111]
miR-214	Ovarian cancer	Stimulation of cell survival and cisplatin resistance	PTEN	[112]
miR-132; miR-212	Pancreatic adenocarcinoma (PDAC)	Stimulation of cell proliferation via β2 adrenergic pathway	Rb1	[113]
miR-375	Gastric cancer	Promotion of carcinogenesis	JAK2 and PDK1	[114]
miR-23b*	Renal cancer cells	Down-regulation of POX (tumor suppressor), increase of HIF signaling	POX	[117]
miR-301	Breast cancer	Promotion of growth, proliferation, invasion, and metastases	FOXF2, BBC3, and PTEN	[118]
miR-675	Colorectal cancer (CRC)	Overexpression of H19 (oncofetal non-coding RNA) in cancer tissues	RB	[119]
miR-296	Brain tumors	Promotion of angiogenesis	HGS	[120]
miR-10b	Human esophageal cancer cells	Promotion of migration and invasion	KLF4	[121]
miR-378	Breast carcinoma	Enhancement of cell survival; reduction of caspase-3 activity; promotion of tumor growth and angiogenesis	Sufu and Fus-1	[122]
miR-372; miR-373	Testicular tumors	Promotion of tumorigenesis in cooperation with RAS	LATS2	[123]

Abbreviations: MITF, microphthalmia-associated transcription factor-M; FOXO3, forkhead box O3; $p27^{Kip1}$, cyclin-dependent kinase inhibitor 1B; PUMA, BCL2 binding component 3; PTEN, PDCD4, phosphatase and tensin homolog; TPM1, tropomyosin 1; P53, tumor protein p53; TGF-B, transforming growth factor, beta 1; CCNG1, cyclin G1; Rb1, retinoblastoma tumor suppressor; JAK2; PDK1, 3′-phosphoinositide dependent protein kinase-1; E2F1, E2F transcription factor 1; Bim, BCL2-like 11 (apoptosis facilitator); CDH1, cadherin 1, type 1, E-cadherin (epithelial); POX, proline oxidase; FOXF2, forkhead box F2; BBC3, BCL2 binding component 3; RB, retinoblastoma 1; HGS, hepatocyte growth factor-regulated tyrosine kinase substrate; KLF4, Kruppel-like factor 4; Sufu, suppressor of fused homolog (Drosophila); Fus-1, tumor suppressor candidate 2; and LATS2, large tumor suppressor, homolog 2 (Drosophila).

Table 3
MiRNAs exhibiting tumor suppressor functions.

MicroRNA	Disease	Biological effects	Target mRNA/pathway	Reference
miR-198	Hepatocellular carcinoma	Inhibition of migration and invasion	HGF/c-MET	[124]
miR-449	Gastric cancer	Inhibition of cell proliferation	GMNN, MET, CCNE2 and SIRT1	[125]
miR-181b	Chronic lymphocytic leukemia	Inhibition of disease progression	Mcl-1 and Bcl-2	[126]
miR-193b	Breast cancer	Alteration of ERalfa signaling, such as steroid synthesis and down-regulation of the ERalfa receptor	AKR1C2, AKR1C1, YWHAZ (14-3-3 family protein)	[127]
miR-218	Gastric cancer	Suppression of tumor metastasis	ROBO1	[128]
miR-126	Non-small cell lung cancer cells	Tumor suppressor genes and involved in development, cell proliferation and cell death, cell migration and blood vessel formation	EGFL7	[129]
miR-15a; miR-16-1	Chronic lymphocytic leukemia	Induction of apoptosis and decrease tumorigenicity	Bcl-2	[130–132]
miR-145; miR-133a; miR-133b	Esophageal squamous cell carcinoma	Inhibition of cell proliferation and cell invasion	FSCN1	[133]
miR-1	Head and neck squamous cell carcinoma (HNSCC)	Inhibition of cell proliferation, invasion, and migration and promotion of apoptosis and cell cycle arrest	TAGLN2	[134]
miR-205	Human prostate	Reduction of cell migration/invasion through down-regulation of protein kinase Cε	CHN1, ErbB3, E2F1, E2F5, ZEB2, PRKCE	[135]
miR-101	Neuroblastoma; bladder transitional cell carcinoma (TCC)	Inhibition of proliferation and clonogenic growth; alteration of global chromatin structure	MYCN EZH2, the catalytic subunit of PRC2	[136]
miR-204	Neuroblastoma	Stimulation of increased sensitivity to cisplatin treatment and promotion of cell survival	TrkB	[137]

Abbreviations: HGF, hepatocyte growth factor (hepapoietin A; scatter factor); c-MET, met proto-oncogene (hepatocyte growth factor receptor); GMNN, geminin, DNA replication inhibitor; CCNE2, cyclin E2; SIRT1, sirtuin 1; Mcl-1, myeloid cell leukemia sequence 1 (BCL2-related); BCL2, B-cell CLL/lymphoma 2; AKR1C1, aldo–keto reductase family 1, member C1 (dihydrodiol dehydrogenase 1; 20-alpha (3-alpha)-hydroxysteroid dehydrogenase); AKR1C2, aldo–keto reductase family 1, member C2 (dihydrodiol dehydrogenase 2; bile acid binding protein; 3-alpha hydroxysteroid dehydrogenase, type III); YWHAZ, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide; ROBO1, Robo1 receptor; EGFL7, EGF-like-domain, multiple 7; FSCN1, fascin homolog 1, actin-bundling protein (*Strongylocentrotus purpuratus*); TAGLN2, transgelin 2; CHN1, chimerin (chimaerin) 1; ErbB3, v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian); E2F1, E2F transcription factor 1; E2F5, E2F transcription factor 5, p130-binding; ZEB2, zinc finger E-box binding homeobox 2; PRKCE, protein kinase C, epsilon; MYCN, v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian); EZH2, enhancer of zeste homolog 2; PRC2, polycomb repressive complex 2; and TrkB, neurotrophic tyrosine kinase, receptor, type 2.

including local invasion, extravasation or initial survival at a distant site, and metastatic colonization [140].

Another interesting feature of the miRNA life was found by studying cancer associated miRNAs in different experimental model systems, i.e. that cancer-specific miRNAs are present in extracellular body fluids, and may play a very important role in the cross-talk between cancer cells and surrounding normal cells [29–31]. The extracellular miRNA are protected by exosome-like structures, small intraluminal vesicles shed from a variety of cells (including cancer cells), with a biogenesis connected with the endosomal sorting complex required for transport (ESCRT) machinery in multivesicular bodies (MVB) [30]. These extracellular structures, originally considered as a "garbage bag" devoted to discard degraded proteins, are now considered of interest as an intercellular communication tool. It is still unclear whether these exosome-associated miRNAs are the result of tumor cell death and lyses, or are actively excreted from tumor cells into the microenvironment. However, this novel secretory machinery of miRNAs may be involved in tumor-associated features, such as enhancement of angiogenesis, increase of cytokine secretion and migration to a pre-metastatic niche [30].

In conclusion, miRNAs are deeply involved in tumor onset and progression [61,62,141,142], so that therapeutic strategies involving miRNA silencing have been proposed [143]. Since miRNAs can behave as tumor suppressor genes, miRNA replacement therapy has been also proposed as a possible therapy of cancer [16].

7. Bioactive molecules altering miR metabolism

Given the role of miRNAs in epigenetic regulation of gene expression, miRNAs have been proposed as possible candidates for drug targeting with the objective of interfering with biological functions, altering the expression of the mRNAs specifically regulated by the targeted miRNAs [15,144–152]. Mature miRNAs can be targeted with short RNA sequences, oligodeoxyribonucleotides (ODNs) and ODN-analogs (such as LNAs). Other molecular targets are Drosha and Dicer (see Figs. 1 and 2). At the transcription level, miRNAs transcription can be altered with strategies able to inhibit (or potentiate) the transcription factors recognizing the miRNA promoter. As already shown in Figs. 1 and 2, different steps of the miRNA biogenesis are suitable for drug targeting, as elsewhere reported [146].

An increasing number of reports has shown that targeting of microRNA biogenesis has a deep impact on specific phenotypes and even on pathological conditions [15,144–146]. It has been demonstrated that miRNAs can be antagonized *in vivo* by oligonucleotides composed of highly affine nucleotide mimics [149–152]. Up to now synthetic oligonucleotides have been used for targeting microRNAs, although with several problems, including delivery and stability.

Only one of the two strands of miR (termed guiding strand) is incorporated into the miRISC complex, and is therefore the ideal target for oligonucleotides designed to inhibit miR functions. Though long mRNA containing multiple target sites could serve as scavenger for miR (called RNA sponge), RNA molecules are not very effective as anti-miR, since they would recreate a dsRNA similar to the miR precursor. Therefore, several modified oligonucleotide analogs have been designed in order to bind to the guiding strand by Watson–Crick base pairing and prevent further processing (Fig. 4). In principle, all derivatives which were proved to be effective in the so called antisense strategy (i.e. targeting of mRNA in order to inhibit gene expression) can also be used to target miR. However, unlike in mRNA targeting, enzymatic degradation by ribonucleases (such as RNaseH) cannot be activated for these short miRs; therefore, only the steric block mechanism can be used, although some degradation by a still unknown mechanism has been reported in some cases.

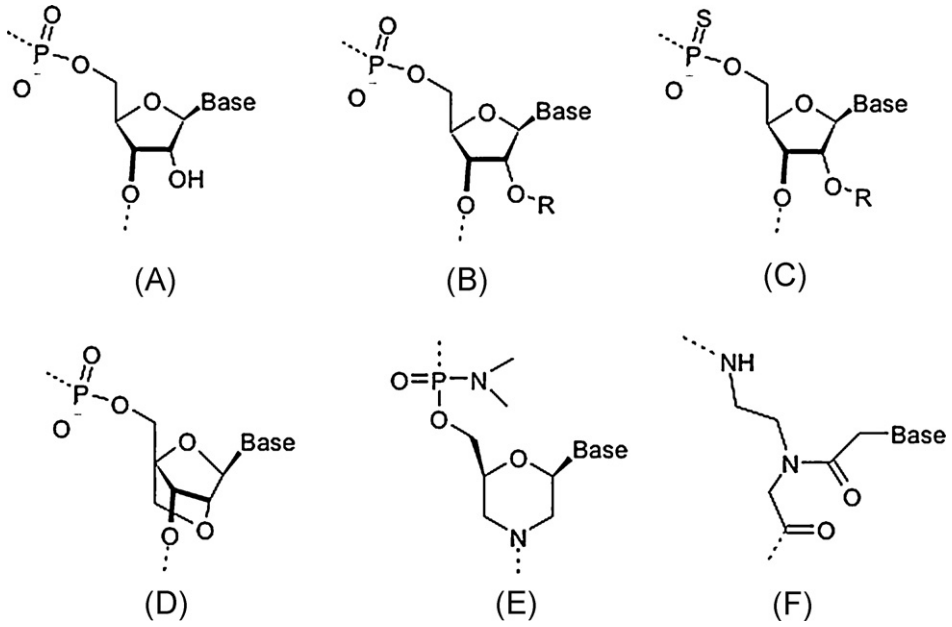

Fig. 4. RNA structure (A) and types of oligonucleotide modification used for miR targeting (B–F); (B) 2′-O-alkyl RNA R = $-CH_3$ or $-CH_2CH_2OCH_3$; (C) phosphorothioate linkage modification; (D) locked nucleic acids (LNAs); (E) morpholino phosphodiamidates (PMO); (F) peptide nucleic acids (PNA).

High stability of the complex formed by the anti-miR agent and the target miRNA is therefore one of the major points. Stability of anti-miR under physiological condition is another important issue; good cellular delivery is also necessary in order to achieve miR inhibition. RNAs are sensitive to both chemical and enzymatic degradation, due to the presence of the 2′-OH group, which can be converted to an internal nucleophile and thus represents RNA "Achille's heel"; therefore, alkylation of the 2′-oxygen in RNA has the effect of producing more stable derivatives with good affinity for complementary miR. Commercially available antago-miRs mainly belong to this class, in particular with methyl and methoxyethyl as alkyl groups; 2′-deoxy-2′-fluoro derivatives have also been used. Phosphorothioate linkages (Fig. 4) can also be introduced in order to increase biostability, in particular resistance to nucleases [150,151]. Locked nucleic acids (LNAs) are RNA analogs in which a methylene bridge has been introduced between the 2′-oxygen and the C4-carbon, thus creating a further ring which strongly constrain the furanoside in a C3′-*endo*-like (i.e. RNA-like) conformation, more effective for RNA recognition. Usually, oligomers containing LNA used in antisense studies have alternated LNA–DNA monomers, whereas those used as anti-miRs are partially made of LNA monomers inserted in a strand composed of 2′-O-methyl RNA units. Interest in these derivatives has been recently boosted by a series of studies showing miR-122 inhibition with subsequent lowering of plasma cholesterol, without signs of toxicity [148,149], and increased resistance to chronic hepatitis C virus (HCV) in primates by targeting miR-122 with LNA, with long-lasting suppression of HCV viremia, and no evidence of viral resistance or side effects in the treated animals. More drastic changes in the backbone of oligonucleotide derivatives such as those of morpholino oligonucleotides (Fig. 4) or peptide nucleic acids (PNAs, see next paragraph) have proven to be effective in miR targeting.

8. Promising approaches using peptide nucleic acids (PNAs)

8.1. Peptide nucleic acids

PNAs (Figs. 4F and 5A) are DNA analogs in which the sugar-phosphate backbone is replaced by N-(2-aminoethyl)glycine units [153–162]. These molecules efficiently hybridize with complementary DNA and RNA, forming double helices with Watson–Crick base pairing [153–167]. In addition, they generate triple helix formation with double stranded DNA and perform strand invasion. They have been proposed for antisense and anti-gene therapy in a number of studies [153–157]. PNAs are promising for RNA recognition, since they have a higher affinity for RNA than for DNA, are more specific, and are resistant to DNases and proteases [154]. PNAs can be modified in order to achieve better performances in terms of cellular permeation, higher affinity, and specificity for the target DNA and RNA sequences [168–173]. While several published papers report the effects of PNAs as anti-gene, anti-mRNA or TF inhibitors, at present, few data are available on the use of PNAs as molecules targeting microRNAs.

8.2. Delivery of PNAs to target cells

One of the most important issues in PNA technology is the uptake by target cells [176–182], the major limit being the low uptake by eukaryotic cells [182–189]. In order to solve this drawback, several approaches have been considered, including the delivery of PNA analogs with liposomes and microspheres [177,181] and the method recently proposed based on the use of a nucleocapsid protein derived from Simian virus 40, for wrapping PNA into pseudovirions, thus facilitating the delivery of the packaged PNA into cells [178]. One possible strategy is to link PNAs to polylysine (K) or a polyarginine (R) tails, based on the observation that these cell-membrane penetrating oligopeptides are able to facilitate uptake of conjugated molecules [188]. Since their discovery, many modifications of the original PNA backbones have been proposed in order to improve performance in term of affinity and specificity. Modification of the PNA backbone with positively charged groups has also been demonstrated to enhance cellular uptake and PNA efficiency [168–173]. Fabani and Gait first delivered anti-miR PNAs by electroporation [174], and in a second experiment, showed that microRNA inhibition can be achieved without the need for transfection or electroporation, by conjugating the PNA to the cell-penetrating peptide R6-Penetratin (R6-pen), or merely by linking to four Lys residues, highlighting the potential of PNA for future therapeutic applications as well as for studying microRNA

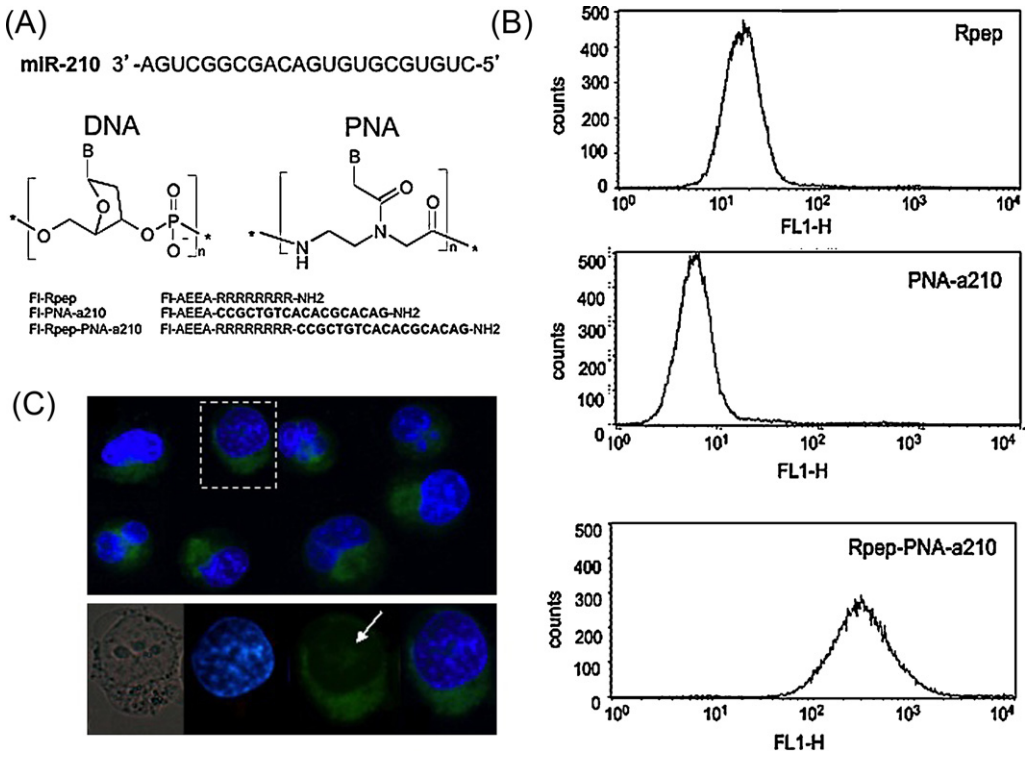

Fig. 5. (A) Sequences of miR-210 and structure of peptide nucleic acids (PNA) (upper part of the panel) and sequences of the employed PNAs and the arginine-rich peptide (Rpep) are shown. Fl: flourescein; AEEA: 2-(2-aminoethoxy)ethoxyacetyl spacer. (B) FACS analysis of K562 cells showing the uptake of fluoresceinated R_8 peptide (R-pep), anti-miR-210 PNA (PNA-a210), and R_8-PNA (R-pep-PNA-a210) after 48 h incubation at a 2 μM concentration. (C) Intracellular distribution. K562 cells were cultured for 48 h with 2 μM of Fl-Rpep-PNA-a210 and then analyzed using a fluorescence microscope. The pictures are the merged analysis of the fluorescence and of the staining of the same cell population with Hoechst 33258 (selectively staining nuclei). Panels at the bottom show a detailed analysis of the cell boxed, demonstrating a cytoplasmic homogenous distribution of Fl-Rpep-PNA-a210.
Modified from Marchelli et al. [188].

function [175]. In a parallel work, Oh et al. described the effectiveness of miR targeting by PNA-peptide conjugates, using a series of cell penetrating peptides (CPP) as carriers, including R6-pen, Tat, a four Lys sequence, and transportan [186]. The best conditions were obtained with cationic peptides, and in particular with the Tat-modified peptide RRRQRRKKRR. In this study, cells were transfected with a plasmid containing a luciferase gene carrying a target site for each miR tested. Inhibition of the miR activity was monitored by expression of the luciferase gene. Inhibition of miR-16, which regulates Bcl-2 expression, and of miR-21 activity could be monitored in this way.

In a recent study we evaluated the activity of a PNA targeting microRNA miR-210, which is firmly associated to hypoxia and is modulated during erythroid differentiation, in leukemic K562 cells [187]. The major conclusion of our study was that a PNA against miR-210 and conjugated with a polyarginine peptide (R-pep-PNA-a210) is efficiently internalized within the target cells (Fig. 5). Fig. 5B shows cellular delivery of fluoresceinated R_8-modified PNA (Rpep-PNA-a210) studied by FACS analysis. In addition, Fig. 5C shows the intracellular distribution of 2 μM of fluoresceinated Rpep-PNA-a210 in K562 cells cultured for 48 h with and then analyzed using a fluorescence microscope. The picture is the merged analysis of the fluorescence and of the staining of the same cell population with Hoechst 33258 (selectively staining nuclei).

8.3. Biological activity of anti-miR PNAs

In a first work, Fabani and Gait [174] demonstrated block of miR-122 activity with a PNA in human and rat liver cells. This block of miR-122 was associated with a decrease of the regulated mRNA, Aldolase A. Furthermore, Fabani et al. [175] demonstrated the activity *in vitro* and *in vivo* of a PNA against miR-155. The inhibition of miR-155 was associated with inhibitory effects on the activity of the target mRNAs Bat5, Sfp1 and Jarid2. Taken together, these data highlight the potential of PNA for future therapeutic applications as well as for studying microRNA functions. In agreement with the role of miR-210 in erythroid differentiation, we found that treatment of K562 with R-pep-PNA-a210 strongly inhibits miR-210 (Fig. 6) and erythroid induction mediated by mithramycin [187,188]. Cellular uptake was found to be crucial in order to obtain biological activity, since the PNA lacking of the poly-arginine tail (PNA-a210), despite being able to hybridize to target nucleotide sequences displayed low activity on cells (Fig. 6) [187,188]. These data support on one hand the hypothesis that miR-210 is involved in erythroid differentiation, and on the other hand that interference with miRNA activity can cause strong biological effects, depending on the role of the microRNA. Further research on the effects of PNAs is needed in different experimental systems (including cellular systems mimicking differentiation) in order to verify if these data can be generalized to other miRNA/mRNA pathways. While the research on anti-miR PNAs is just started, pre-clinical results are expected in the near future to sustain the hypothesis that miRNA-targeted molecules based on PNAs can be successfully applied to treat human diseases. An example was recently reported by Yan et al. [189], who address the potential effects of PNA-antimiR-21 *in vivo* on the growth of breast cancer cells. In their experiments, MCF-7 cells treated with PNA-antimiR-21 or PNA-control were subcutaneously injected into female nude mice (eight animals per treatment). Detectable tumor masses were seen in only 5/8 of mice in the MCF-7/PNA-antimiR-21 group, while much larger tumors were detected in all mice in the MCF-7/PNA-control group. Both tumor weight and number

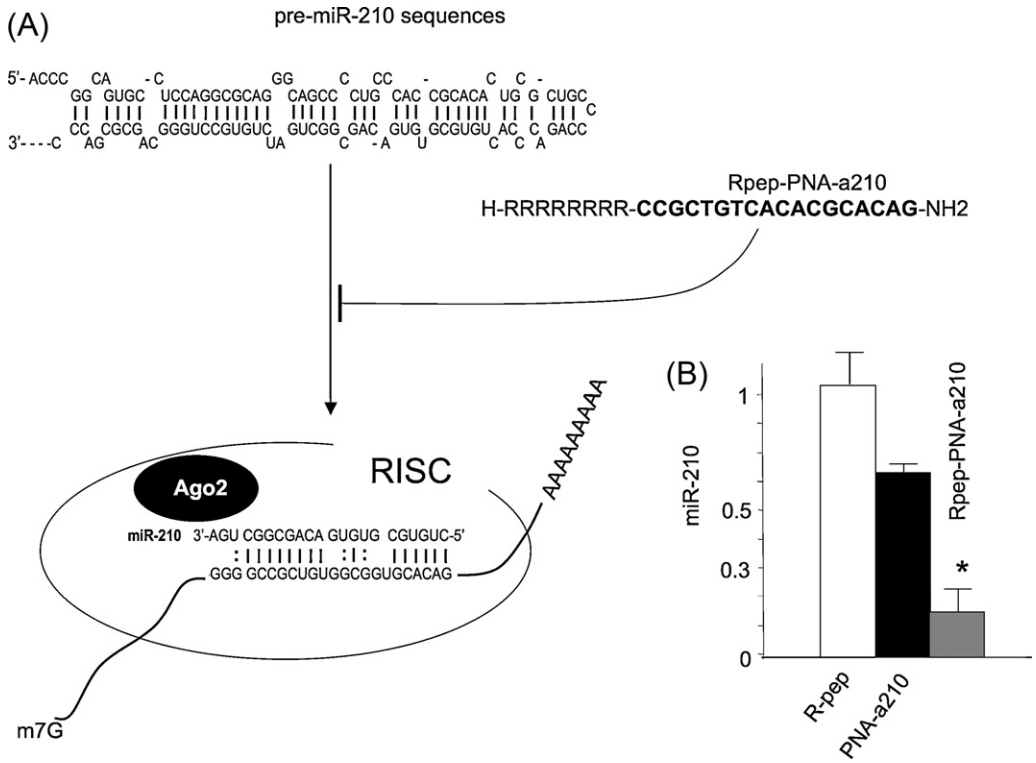

Fig. 6. Scheme of the PNA-mediated interference of miR/mRNA interactions (A) and quantification by real time PCR of miR-210 in MTH-induced K562 cells cultured in the presence of Rpep, PNA-a210, Rpep-PNA-a210 (B). PNAs were administrated at the concentration of 500 nM and analysis of miR-210 was performed using untreated cells as control. The data represent the average ± S.D. from 3 different experiments. The cells were seeded at 30,000/ml of concentration and 30 nM MTH added at first days of culture after PNA treatment.
Modified from Marchelli et al. [188].

showed that MCF-7/PNA-control cells formed larger tumors more rapidly than MCF-7/PNA-antimiR-21 cells in nude mice.

9. MicroRNA therapeutics and clinical trials

On the basis of the studies demonstrating that microRNAs are promising candidates for drug targeting, many activities aiming at developing possible reagents for therapy and diagnostics are in progress in order to bring this research to industrial exploitation and to clinical settings [71,190]. At present, miRNAs are likely to be used as biomarkers in clinical settings sooner than as therapeutic reagents. This is evident by looking at patents and clinical trials (Table 4). As far as clinical trials (www.clinicaltrials.com), only few are on miRNA therapeutics (for instance a Phase I trial is active on targeting miR-122 for therapy of HCV-infection), while the majority of them are focused on miRNA diagnostics. In any case, several companies have already launched their activities, including research activities for producing tools for miRNA diagnostics and therapeutics in their objectives [191], such as Exiqon A/S, that offers locked nucleic acid (LNA) technology, Febit that employs a

Table 4
Selected companies involved in miRNA research for drug development.

Company	Focus and pipelines	Patents
Mirna Therapeutics (http://www.mirnatherapeutics.com/)	miRNA-directed oncology therapies on non-small lung cancer (NSCLC), advanced prostate cancer, other solid tumors	Composition for the *in vivo* delivery of RNAi agents (EP2306978-A2) Methods and compositions involving miRNAs in cancer stem cells (WO2010056737-A2)
Regulus Therapeutics (http://www.regulusrx.com/)	Advancement of microRNA therapeutics across several areas, including fibrosis, hepatitis C (HCV) infection, immuno-inflammatory diseases, metabolic diseases, and oncology (liver cancer)	Oligomeric compounds and compositions for use in modulation of pri-miRNAs (US2010249215-A1) MicroRNA detection (US2009236225-A1) Targeting microRNAs for the treatment of liver cancer (US2010267814-A1) Antisense compounds having enhanced anti-microRNA activity (US2009203893-A1)
Sirna Therapeutics (http://www.sirna.com/)	Oligonucleotide optimization for improving resistance to degradation, reducing immunostimulation, enhancing Ago2/RISC incorporation and potency, increasing chemical stability, improving target specificity	RNA interference-mediated inhibition of vascular endothelium growth factor and vascular endothelium growth factor receptor gene expression using short interfering nucleic acid (siNA) (JP2009000105-A)
Alnylam Pharmaceuticals (http://www.alnylam.com/)	RNAi for the targeting of disease-causing genes in the genome. Treatment of genetically defined diseases, including transthyretin-mediated amyloidosis (ATTR), severe hypercholesterolemia, refractory anemia, respiratory syncytial virus (RSV) infection, liver cancers, and Huntington's disease	Chemically modified oligonucleotides for use in modulating microRNA and uses thereof (US2010222413-A1)

Table 4 (*Continued*)

Company	Focus and pipelines	Patents
Santaris Pharma (http://www.santaris.com/)	Development of LNA Drug Platforms and Drug Discovery. Delivery of potent single-stranded LNA-based drug candidates for a range of diseases including metabolic disorders, infectious and inflammatory diseases, cancer and rare genetic disorders	Pharmaceutical composition comprising anti-miRNA antisense oligonucleotides (KR20080108154-A) Micro-RNA mediated modulation of colony stimulating factors (WO2010012667-A1) Pharmaceutical compositions for treatment of HCV patients that are non-responders to interferon (WO2010122538-A1) Pharmaceutical compositions for treatment of microRNA related disease (US2011077288-A1)
Rosetta Genomics (http://www.rosettagenomics.com/)	Development of microRNA-based diagnostic tests for cancer. Prediction of the risk of a superficial bladder cancer to become invasive. Development of tests able to classify tumors. Development of microRNA-based therapeutics focusing on hepatocellular carcinoma, the most common type of liver cancer	Small molecules modulating activity of microRNA oligonucleotides and microRNA targets and uses thereof (US7687616-B1) MicroRNA and uses thereof (CN101031657-A) MicroRNAs expression signature for determinations of tumors origin (WO2009066291-A2) Compositions and methods for the treatment of glioblastoma (WO2010023658-A2)(a) Methods for detecting an increased susceptibility to cancer (WO2010061396-A1)
ActiGenics (http://www.actigenics.com/)	Development of molecules for identification of pathological targets employing bioinformatic platforms. Design of miRNAs to inhibit a set of functionally interrelated messenger RNAs in order to change gene expression	Methods for the identification of microRNA and their applications in research and human health (EP1783645-A1)
Isis Pharmaceuticals (http://www.isispharm.com/)	RNA chemistry and methods of manipulation for applications in the following fields: cardiovascular, metabolic, cancer, rare diseases, neurodegenerative diseases, inflammation	Methods for use in modulating miR-122A (EP1931782-A2)
Miragen Therapeutics (http://www.miragentherapeutics.com/)	Development of AntimiR Programs (indications: chronic heart failure, polycytemia vera) and PromiR Programs (indication: cardiac fibrosis)	Chemical modification motifs for miRNA inhibitors and mimetics (WO2101144485-A1) Lipophilic polynucleotide conjugates (WO210129672)

Source: http://worldwide.espacenet.com/. In the case of multiple patents with similar claims, only one representative patent is shown. (a) Similar patents have been proposed for lymphoma (WO2010018563-A2), and kidney (WO2010016064-A2), colorectal (WO2010004562-A2), lung (WO2009153775-A2), gastric (WO2009147656-A1), colon (WO2010058393-A2), prostate (WO2011024157-A1), renal (WO2011039757-A2), ovarian (US2011105596-A1) and bladder urothelial (US2011143959-A1) cancers.

unique onchip labeling method for miRNA expression profiling with sensitivity [30]. Exosome Diagnostics, as the name suggests, is focused on utilizing secretory tumor exosomes as diagnostic markers [30]. As far as companies involved in miRNA therapeutics, Table 4 shows a partial list of companies who have included miRNA anti-sense and miRNA mimics in their pipelines [191].

10. Conclusions and perspectives

MicroRNAs are promising candidates for drug targeting: the aim is to develop possible molecular systems for experimental therapy of human pathologies in which microRNAs appears to be deeply involved. PNAs are bioactive molecules and promising tools for the inhibition of miRNA activity. This effect can be very important in obtaining gene modulation in a simple way, with major applications in gene therapy and in drug development. The issue of delivering PNA to their targets is still open, although efficient strategies have already been described, including conjugation with carrier peptides and backbone modifications.

The high affinity of PNA for RNA and the strong chemical and enzymatic stability of these compounds (especially the backbone-modified version) make them ideal candidates as miRNA inhibitors with long-lasting effects.

Furthermore, the possibility to introduce functional groups along the chain of the PNA strand by chemical synthesis allows to envisage strategies in which the PNA can be endowed of catalytic sites, thus leading to molecules not only capable of binding, but also of cleaving nucleotide sequences, leading to miRNA specific nuclease models.

Acknowledgments

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References

[1] Costa FF. Epigenomics in cancer management. Cancer Manag Res 2010;2:255–65.
[2] Filipowicz W, Jaskiewicz L, Kolb FA, Pillai RS. Post-transcriptional gene silencing by siRNAs and miRNAs. Curr Opin Struct Biol 2005;15:331–41.
[3] He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet 2010;5:522–31.
[4] Kozomara A, Griffiths-Jones S. miRBase: integrating microRNA annotation and deep-sequencing data. Nucleic Acids Res 2010;39:D152–7.
[5] Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 2010;11:597–610.
[6] Sontheimer EJ, Carthew RW. Silence from within: endogenous siRNAs and miRNAs. Cell 2005;122:9–12.
[7] Subramanian S, Steer CJ. MicroRNAs as gatekeepers of apoptosis. J Cell Physiol 2010;223:89–98.
[8] Wang YM, Blelloch R. Cell cycle regulation by microRNAs in embryonic stem cells. Cancer Res 2010;69:4093–6.
[9] Alvarez-Garcia I, Miska EA. MicroRNA functions in animal development and human disease. Development 2005;132:4653–62.
[10] Taccioli C, Fabbri E, Visone R, Volinia S, Calin GA, Fong LY, et al. UCbase & miRfunc: a database of ultraconserved sequences and microRNA function. Nucleic Acids Res 2009;37:D41–8.
[11] Tsai LM, Yu D. MicroRNAs in common diseases and potential therapeutic applications. Clin Exp Pharmacol Physiol 2010;37:102–7.
[12] Tiscornia G, Izpisúa Belmonte JC. MicroRNAs in embryonic stem cell function and fate. Genes Dev 2010;24:2732–41.
[13] Bianchi N, Zuccato C, Lampronti I, Borgatti M, Gambari R. Expression of miR-210 during erythroid differentiation and induction of γ-globin gene expression. BMB Rep 2009;8:493–9.
[14] Hemida MG, Ye X, Thair S, Yang D. Exploiting the therapeutic potential of microRNAs in viral diseases: expectations and limitations. Mol Diagn Ther 2010;14:271–82.

[15] Kota SK, Balasubramanian S. Cancer therapy via modulation of micro RNA levels: a promising future. Drug Discov Today 2010;15:733–40.
[16] Bader AG, Brown D, Winkler M. The promise of microRNA replacement therapy. Cancer Res 2010;70:7027–30.
[17] Sibley CR, Seow Y, Wood MJ. Novel RNA-based strategies for therapeutic gene silencing. Mol Ther 2010;18:466–76.
[18] Ofir M, Hacohen D, Ginsberg D. miR-15 and miR-16 are direct transcriptional targets of E2F1 that limit E2F-induced proliferation by targeting cyclin E. Mol Cancer Res 2011;9:440–7.
[19] Woods K, Thomson JM, Hammond SM. Direct regulation of an oncogenic micro-RNA cluster by E2F transcription factors. J Biol Chem 2007;282: 2130–4.
[20] Wang K, Li PF. Foxo3a regulates apoptosis by negatively targeting miR-21. J Biol Chem 2010;285:16958–66.
[21] Rainer J, Ploner C, Jesacher S, Ploner A, Eduardoff M, Mansha M, et al. Glucocorticoid-regulated microRNAs and mirtrons in acute lymphoblastic leukemia. Leukemia 2009;23:746–52.
[22] Gerin I, Clerbaux LA, Haumont O, Lanthier N, Das AK, Burant CF, et al. Expression of miR-33 from an SREBP2 intron inhibits cholesterol export and fatty acid oxidation. J Biol Chem 2010;285:33652–61.
[23] Berezikov E, Chung WJ, Willis J, Cuppen E, Lai EC. Mammalian mirtron genes. Mol Cell 2007;28:328–36.
[24] Ma N, Wang X, Qiao Y, Li F, Hui Y, Zou C, et al. Coexpression of an intronic microRNA and its host gene reveals a potential role for miR-483-5p as an IGF2 partner. Mol Cell Endocrinol 2011;333:96–101.
[25] Levy C, Khaled M, Iliopoulos D, Janas MM, Schubert S, Pinner S, et al. Intronic miR-211 assumes the tumor suppressive function of its host gene in melanoma. Mol Cell 2010;40:841–9.
[26] Hinske LC, Galante PA, Kuo WP, Ohno-Machado L. A potential role for intragenic miRNAs on their hosts' interactome. BMC Genomics 2010;11:533.
[27] Cao G, Huang B, Liu Z, Zhang J, Xu H, Xia W, et al. Intronic miR-301 feedback regulates its host gene, ska2, in A549 cells by targeting MEOX2 to affect ERK/CREB pathways. Biochem Biophys Res Commun 2010;396:978–82.
[28] Xi S, Yang M, Tao Y, Xu H, Shan J, Inchauste S, et al. Cigarette smoke induces C/EBP-β-mediated activation of miR-31 in normal human respiratory epithelia and lung cancer cells. PLoS One 2010;5:e13764.
[29] Kosaka N, Iguchi H, Yoshioka Y, Takeshita F, Matsuki Y, Ochiya T. Secretory mechanisms and intercellular transfer of microRNAs in living cells. J Biol Chem 2010;285:17442–5.
[30] Iguchi H, Kosaka N, Ochiya T. Versatile applications of microRNA in anticancer drug discovery: from therapeutics to biomarkers. Curr Drug Discov Technol 2010;7:95–105.
[31] Kosaka N, Iguchi H, Ochiya T. Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. Cancer Sci 2010;101:2087–92.
[32] Cuccato G, Polynikis A, Siciliano V, Graziano M, di Bernardo M, di Bernardo D. Modeling RNA interference in mammalian cells. BMC Syst Biol 2011;5:19.
[33] Moser JJ, Fritzler MJ. The microRNA and messengerRNA profile of the RNA-induced silencing complex in human primary astrocyte and astrocytoma cells. PLoS One 2010;5:e13445.
[34] Yoda M, Kawamata T, Paroo Z, Ye X, Iwasaki S, Liu Q, et al. ATP-dependent human RISC assembly pathways. Nat Struct Mol Biol 2010;17:17–23.
[35] Choe J, Cho H, Lee HC, Kim YK. MicroRNA/Argonaute 2 regulates nonsense-mediated messenger RNA decay. EMBO Rep 2010;11:380–6.
[36] Bracken CP, Szubert JM, Mercer TR, Dinger ME, Thomson DW, Mattick JS, et al. Global analysis of the mammalian RNA degradome reveals widespread miRNA-dependent and miRNA-independent endonucleolytic cleavage. Nucleic Acids Res 2011;39:5658–68.
[37] Huntzinger E, Izaurralde E. Gene silencing by microRNAs: contributions of translational repression and mRNA decay. Nat Rev Genet 2011;12:99–110.
[38] Felli N, Fontana L, Pelosi E, Botta R, Bonci D, Facchiano F, et al. Proc Natl Acad Sci USA 2005;102:18081–6.
[39] Choong ML, Yang HH, McNiece I. MicroRNA expression profiling during human cord blood-derived CD34 cell erythropoiesis. Exp Hematol 2007;35:551–64.
[40] Georgantas 3rd RW, Hildreth R, Morisot S, Alder J, Liu CG, Heimfeld S, et al. Proc Natl Acad Sci USA 2007;104:2750–5.
[41] Sankaran VG, Menne TF, Šćepanović D, Vergilio JA, Ji P, Kim J, et al. MicroRNA-15a and -16-1 act via MYB to elevate fetal hemoglobin expression in human trisomy 13. Proc Natl Acad Sci USA 2011;108:1519–24.
[42] Huang X, Gschweng E, Van Handel B, Cheng D, Mikkola HK, Witte ON. Regulated expression of microRNAs-126/126* inhibits erythropoiesis from human embryonic stem cells. Blood 2011;117:2157–65.
[43] Grabher C, Payne EM, Johnston AB, Bolli N, Lechman E, Dick JE, et al. Zebrafish microRNA-126 determines hematopoietic cell fate through c-Myb. Leukemia 2011;25:506–14.
[44] Fibach E, Bianchi N, Borgatti M, Prus E, Gambari R. Mithramycin induces fetal hemoglobin production in normal and thalassemic human erythroid precursor cells. Blood 2003;102:1276–81.
[45] Fasanaro P, D'Alessandra Y, Di Stefano V, Melchionna R, Romani S, Pompilio G, et al. MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3. J Biol Chem 2008;283:15878–83.
[46] Camps C, Buffa FM, Colella S, Moore J, Sotiriou C, Sheldon H, et al. hsa-miR-210 is induced by hypoxia and is an independent prognostic factor in breast cancer. Clin Cancer Res 2008;14:1340–8.
[47] Giannakakis A, Sandaltzopoulos R, Greshock J, Liang S, Huang J, Hasegawa K, et al. miR-210 links hypoxia with cell cycle regulation and is deleted in human epithelial ovarian cancer. Cancer Biol Ther 2008;7:255–64.
[48] Kulshreshtha R, Davuluri RV, Calin GA, Ivan M. A microRNA component of the hypoxic response. Cell Death Differ 2008;15:667–71.
[49] Rogers HM, Yu X, Wen J, Smith R, Fibach E, Noguch CT. Hypoxia alters progression of the erythroid program. Exp Hematol 2008;36:17–27.
[50] Narayan AD, Ersek A, Campbell TA, Colon DM, Pixley JS, Zanjani ED. The effect of hypoxia and stem cell source on haemoglobin switching. Br J Haematol 2005;128:562–70.
[51] Alter BP. Fetal erythropoiesis in stress hematopoiesis. Exp Hematol 1979;7:200–9.
[52] Kosaka N, Sugiura K, Yamamoto Y, Yoshioka Y, Miyazaki H, Komatsu N, et al. Identification of erythropoietin-induced microRNAs in haematopoietic cells during erythroid differentiation. Br J Haematol 2008;142:293–300.
[53] Gambari R, Fibach E. Medicinal chemistry of fetal hemoglobin inducers for treatment of beta-thalassemia. Curr Med Chem 2007;14:199–212.
[54] Lampronti I, Bianchi N, Borgatti M, Fibach E, Prus E, Gambari R. Accumulation of gamma-globin mRNA in human erythroid cells treated with angelicin. Eur J Haematol 2003;71:189–95.
[55] Gabbianelli M, Testa U, Morsilli O, Pelosi E, Saulle E, Petrucci E, et al. Mechanism of human Hb switching: a possible role of the kit receptor/miR 221-222 complex. Haematologica 2010;95:1253–60.
[56] Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006;6:857–66.
[57] Cho WCS. OncomiRs: the discovery and progress of microRNAs in cancers. Mol Cancer 2006;6:60.
[58] Garzon R, Croce CM. MicroRNAs in normal and malignant hematopoiesis. Curr Opin Hematol 2008;15:352–8.
[59] Fu SW, Chen L, Man YG. miRNA biomarkers in breast cancer detection and management. J Cancer 2011;2:116–22.
[60] Nana-Sinkam SP, Hunter MG, Nuovo GJ, Schmittgen TD, Gelinas R, Galas D, et al. Integrating the MicroRNome into the study of lung disease. Am J Respir Crit Care Med 2009;179:4–10.
[61] Edmonds MD, Hurst DR, Welch DR. Linking metastasis suppression with metastamiR regulation. Cell Cycle 2009;8:2673–5.
[62] Hurst DR, Edmonds MD, Welch DR. Metastamir: the field of metastasis-regulatory microRNA is spreading. Cancer Res 2009;69:7495–8.
[63] Sugatani T, Vacher J, Hruska KA. A microRNA expression signature of osteoclastogenesis. Blood 2011;117:3648–57.
[64] Li JY, Yong TY, Michael MZ, Gleadle JM. The role of microRNAs in kidney disease. Nephrology 2010;15:599–608.
[65] Saugstad JA. MicroRNAs as effectors of brain function with roles in ischemia and injury, neuroprotection, and neurodegeneration. J Cereb Blood Flow Metab 2010;30:1564–76.
[66] Shafi G, Aliya N, Munshi A. MicroRNA signatures in neurological disorders. Can J Neurol Sci 2010;37:177–85.
[67] Kolfschoten IG, Roggli E, Nesca V, Regazzi R. Role and therapeutic potential of microRNAs in diabetes. Diabetes Obes Metab 2009;11:118–29.
[68] Taft RJ, Pang KC, Mercer TR, Dinger M, Mattick JS. Non-coding RNAs: regulators of disease. J Pathol 2010;220:126–39.
[69] Corsten MF, Miranda R, Kasmieh R, Krichevsky AM, Weissleder R, Shah K. MicroRNA-21 knockdown disrupts glioma growth in vivo and displays synergistic cytotoxicity with neural precursor cell-delivered S-TRAIL in human gliomas. Cancer Res 2007;67:8994–9000.
[70] Dai R, Ahmed SA. MicroRNA, a new paradigm for understanding immunoregulation, inflammation, and autoimmune diseases. Transl Res 2011;157: 163–79.
[71] Wu WK, Law PT, Lee CW, Cho CH, Fan D, Wu K, et al. MicroRNA in colorectal cancer: from benchtop to bedside. Carcinogenesis 2011;32: 247–53.
[72] Büssing I, Slack FJ, Grosshans H. Let-7 microRNAs in development, stem cells and cancer. Trends Mol Med 2008;14:400–9.
[73] Heneghan HM, Miller N, Kerin MJ. Role of microRNAs in obesity and the metabolic syndrome. Obes Rev 2010;11:354–61.
[74] Xie H, Sun L, Lodish HF. Targeting microRNAs in obesity. Expert Opin Ther Targets 2009;13:1227–38.
[75] Ferland-McCollough D, Ozanne SE, Siddle K, Willis AE, Bushell M. The involvement of microRNAs in Type 2 diabetes. Biochem Soc Trans 2010;3: 1565–70.
[76] Kuhn S, Johnson SL, Furness DN, Chen J, Ingham N, Hilton JM, et al. miR-96 regulates the progression of differentiation in mammalian cochlear inner and outer hair cells. Proc Natl Acad Sci USA 2011;108:2355–60.
[77] Kerr TA, Korenblat KM, Davidson NO. MicroRNAs and liver disease. Transl Res 2011;157:241–52.
[78] Bravo JA, Dinan TG. MicroRNAs: a novel therapeutic target for schizophrenia. Curr Pharm Des 2011;17:176–88.
[79] Abu-Elneel K, Liu T, Gazzaniga FS, Nishimura Y, Wall DP, Geschwind DH, et al. Heterogeneous dysregulation of microRNAs across the autism spectrum. Neurogenetics 2008;9:153–61.
[80] Hansen T, Olsen L, Lindow M, Jakobsen KD, Ullum H, Jonsson E, et al. Brain expressed microRNAs implicated in schizophrenia etiology. PLoS One 2007;2:e873.
[81] Xu N, Brodin P, Wei T, Meisgen F, Eidsmo L, Nagy N, et al. MiR-125b, a microRNA downregulated in psoriasis, modulates keratinocyte proliferation by targeting FGFR2. J Invest Dermatol 2011;131:1521–9.

[82] Sonkoly E, Wei T, Janson PC, Sääf A, Lundeberg L, Tengvall-Linder M, et al. MicroRNAs: novel regulators involved in the pathogenesis of psoriasis? PLoS One 2007;2(July):e610.
[83] Fiore R, Schratt G. MicroRNAs in synapse development: tiny molecules to remember. Expert Opin Biol Ther 2007;7:1823–31.
[84] Guglielmelli P, Tozzi L, Bogani C, Iacobucci I, Ponziani V, Martinelli G, et al. Over-expression of microRNA-16-2 contributes to the abnormal erythropoiesis in polycythemia vera. Blood 2011;117:6923–7.
[85] Bruchova H, Yoon D, Agarwal AM, Mendell J, Prchal JT. Regulated expression of microRNAs in normal and polycythemia vera erythropoiesis. Exp Hematol 2007;35:1657–67.
[86] Noorbakhsh F, Ramachandran R, Barsby N, Ellestad KK, LeBlanc A, Dickie P, et al. MicroRNA profiling reveals new aspects of HIV neurodegeneration: caspase-6 regulates astrocyte survival. FASEB J 2010;24:1799–812.
[87] Hariharan M, Scaria V, Pillai B, Brahmachari SK. Targets for human encoded microRNAs in HIV genes. Biochem Biophys Res Commun 2005;337:1214–8.
[88] Lu M, Zhang Q, Deng M, Miao J, Guo Y, Gao W, et al. An analysis of human microRNA and disease associations. PLoS One 2008;3:e3420.
[89] Wang D, Wang J, Lu M, Song F, Cui Q. Inferring the human microRNA functional similarity and functional network based on microRNA-associated diseases. Bioinformatics 2010;26:1644–50.
[90] Jordan SD, Krüger M, Willmes DM, Redemann N, Wunderlich TF, Brönneke HS, et al. Obesity-induced overexpression of miRNA-143 inhibits insulin-stimulated AKT activation and impairs glucose metabolism. Nat Cell Biol 2011;13:434–46.
[91] Martinelli R, Nardelli C, Pilone V, Buonomo T, Liguori R, Castanò I, et al. miR-519d overexpression is associated with human obesity. Obesity 2010;18:2170–6.
[92] Ouaamari AE, Baroukh N, Martens GA, Patricia Lebrun P, Pipeleers D, Obberghen E. miR-375 targets 3′-phosphoinositide-dependent protein kinase-1 and regulates glucose-induced biological responses in pancreatic β-cells. Diabetes 2008;57:2708–17.
[93] Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P. Modulation of hepatitis C virus RNA abundance by a liver-specific microRNA. Science 2005;309:1577.
[94] Kapinas K, Kessler C, Ricks T, Gronowicz G, Delany AM. miR-29 modulates Wnt signaling in human osteoblasts through a positive feedback loop. J Biol Chem 2010;285:25221–3.
[95] Sonkoly E, Wei T, Janson PC, Sääf A, Lundeberg L, Tengvall-Linder M, et al. MicroRNAs: novel regulators involved in the pathogenesis of psoriasis? PLoS One 2007;2:e610.
[96] van Almen GC, Verhesen W, van Leeuwen RE, van de Vrie M, Eurlings C, Schellings MW, et al. MicroRNA-18 and microRNA-19 regulate CTGF and TSP-1 expression in age-related heart failure. Aging Cell 2011. doi: 10.1111/j.1474-9726.2011.00714.x.
[97] Thum T, Gross C, Fiedler J, Fischer T, Kissler S, Bussen M, et al. MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. Nature 2008;456:980–4.
[98] Wang WX, Rajeev BW, Stromberg AJ, Ren N, Tang G, Huang Q, et al. The expression of microRNA miR-107 decreases early in Alzheimer's disease and may accelerate disease progression through regulation of beta-site amyloid precursor protein-cleaving enzyme 1. J Neurosci 2008;28:1213–23.
[99] Cordes KR, Sheehy NT, White MP, Berry EC, Morton SU, Muth AN, et al. miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. Nature 2009;460:705–10.
[100] Mencía A, Modamio-Høybjør S, Redshaw N, Morín M, Mayo-Merino F, Olavarrieta L, et al. Mutations in the seed region of human miR-96 are responsible for nonsyndromic progressive hearing loss. Nat Genet 2009;41:609–13.
[101] Lewis MA, Quint E, Glazier AM, Fuchs H, De Angelis MH, Langford C, et al. An ENU-induced mutation of miR-96 associated with progressive hearing loss in mice. Nat Genet 2009;41:614–8.
[102] Segura MF, Hanniford D, Menendez S, Reavie L, Zou X, Alvarez-Diaz S, et al. Aberrant miR-182 expression promotes melanoma metastasis by repressing FOXO3 and microphthalmia-associated transcription factor. Proc Natl Acad Sci USA 2009;106:1814–9.
[103] Sredni ST, Bonaldo Mde F, Costa FF, Huang CC, Hamm CA, Rajaram V, et al. Upregulation of mir-221 and mir-222 in atypical teratoid/rhabdoid tumors: potential therapeutic targets. Childs Nerv Syst 2010;26:279–83.
[104] Zhang CZ, Zhang JX, Zhang AL, Shi ZD, Han L, Jia ZF, et al. MiR-221 and miR-222 target PUMA to induce cell survival in glioblastoma. Mol Cancer 2010;9:229.
[105] Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA 2006;103:2257–61.
[106] Frankel LB, Christoffersen NR, Jacobsen A, Lindow M, Krogh A, Lund AH. Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. J Biol Chem 2008;283:1026–33.
[107] Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, et al. A microRNA signature associated with prognosis and progression inchronic lymphocytic leukemia. N Engl J Med 2005;353:1793–801.
[108] Garzon R, Volinia S, Liu CG, Fernandez-Cymering C, Palumbo T, Pichiorri F, et al. MicroRNA signatures associated with cytogenetics and prognosis in acute myeloid leukemia. Blood 2008;111:3183–9.
[109] Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Res 2005;65:6029–33.
[110] Papagiannakopoulos T, Shapiro A, Kosik KS. MicroRNA-21 targets a network of key tumor-suppressive pathways in glioblastoma cells. Cancer Res 2008;68:8164–72.
[111] Gramantieri L, Ferracin M, Fornari F, Veronese A, Sabbioni S, Liu CG, et al. Cyclin G1is a target of miR-122a, a microRNA frequently down-regulated in human hepatocellular carcinoma. Cancer Res 2007;67:6092–9.
[112] Yang H, Kong W, He L, Zhao JJ, O'Donnell JD, Wang J, et al. MicroRNA expression profiling in human ovarian cancer: miR-214 induces cell survival and cisplatin resistance by targeting PTEN. Cancer Res 2008;68:425–33.
[113] Park JK, Henry JC, Jiang J, Esau C, Gusev Y, Lerner MR, et al. miR-132 and miR-212 are increased in pancreatic cancer and target the retinoblastoma tumor suppressor. Biochem Biophys Res Commun 2011;406:518–23.
[114] Xu Y, Deng Y, Yan X, Zhou T. Targeting miR-375 in gastric cancer. Expert Opin Ther Targets 2011;15:961–72.
[115] Xiao C, Srinivasan L, Calado DP, Patterson HC, Zhang B, Wang J, et al. Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes. Nat Immunol 2008;9:405–14.
[116] Ma L, Young J, Prabhala H, Pan E, Mestdagh P, Muth D, et al. miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. Nat Cell Biol 2010;12:247–56.
[117] Liu W, Zabirnyk O, Wang H, Shiao YH, Nickerson ML, Khalil S, et al. miR-23b targets proline oxidase, a novel tumor suppressor protein in renal cancer. Oncogene 2010;29:4914–24.
[118] Shi W, Gerster K, Alajez NM, Tsang J, Waldron L, Pintilie M, et al. MicroRNA-301 mediates proliferation and invasion in human breast cancer. Cancer Res 2011;71:2926–37.
[119] Tsang WP, Ng EK, Ng SS, Jin H, Yu J, Sung JJ, et al. Oncofetal H19-derived miR-675 regulates tumor suppressor RB in human colorectal cancer. Carcinogenesis 2010;31:350–8.
[120] Wurdinger T, Tannous BA, Saydam O, Skog J, Grau S, Soutschek J, et al. miR-296 regulates growth factor receptor overexpression in angiogenic endothelial cells. Cancer Cell 2008;14:382–93.
[121] Tian Y, Luo A, Cai Y, Su Q, Ding F, Chen H, et al. MicroRNA-10b promotes migration and invasion through KLF4 in human esophageal cancer cell lines. J Biol Chem 2010;285:7986–94.
[122] Lee DY, Deng Z, Wang CH, Yang BB. MicroRNA-378 promotes cell survival, tumor growth, and angiogenesis by targeting SuFu and Fus-1 expression. Proc Natl Acad Sci USA 2007;104:20350–5.
[123] Voorhoeve PM, le Sage C, Schrier M, Gillis AJ, Stoop H, Nagel R, et al. A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. Adv Exp Med Biol 2007;604:17–46.
[124] Tan S, Li R, Ding K, Lobie PE, Zhu T. miR-198 inhibits migration and invasion of hepatocellular carcinoma cells by targeting the HGF/c-MET pathway. FEBS Lett 2011;585:2229–34.
[125] Bou Kheir T, Futoma-Kazmierczak E, Jacobsen A, Krogh A, Bardram L, Hother C, et al. miR-449 inhibits cell proliferation and is down-regulated in gastric cancer. Mol Cancer 2011;10:29.
[126] Visone R, Veronese A, Rassenti LZ, Balatti V, Pearl DK, Acunzo M, et al. MiR-181b is a biomarker of disease progression in chronic lymphocytic leukemia. Blood 2011. doi: 10.1182/blood-2011-01-333484.
[127] Leivonen SK, Rokka A, Ostling P, Kohonen P, Corthals GL, Kallioniemi O, et al. Identification of miR-193b targets in breast cancer cells and systems biological analysis of their functional impact. Mol Cell Proteomics 2011. doi: 10.1074/mcp.
[128] Tie J, Pan Y, Zhao L, Wu K, Liu J, Sun S, et al. MiR-218 inhibits invasion and metastasis of gastric cancer by targeting the Robo1 receptor. PLoS Genet 2010;6:e1000879.
[129] Sun Y, Bai Y, Zhang F, Wang Y, Guo Y, Guo L. miR-126 inhibits non-small cell lung cancer cells proliferation by targeting EGFL7. Biochem Biophys Res Commun 2010;391:1483–9.
[130] Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci USA 2005;102:13944–9.
[131] Linsley PS, Schelter J, Burchard J, Kibukawa M, Martin MM, Bartz SR, et al. Transcripts targeted by the microRNA-16 family cooperatively regulate cell cycle progression. Mol Cell Biol 2007;27:2240–52.
[132] Calin GA, Cimmino A, Fabbri M, Ferracin M, Wojcik SE, Shimizu M, et al. MiR-15a and miR-16-1 cluster functions in human leukemia. Proc Natl Acad Sci USA 2008;105:5166–71.
[133] Kano M, Seki N, Kikkawa N, Fujimura L, Hoshino I, Akutsu Y, et al. miR-145, miR-133a and miR-133b: tumor-suppressive miRNAs target FSCN1 in esophageal squamous cell carcinoma. Int J Cancer 2010;127:2804–14.
[134] Nohata N, Sone Y, Hanazawa T, Fuse M, Kikkawa N, Yoshino H, et al. miR-1 as a tumor suppressive microRNA targeting TAGLN2 in head and neck squamous cell carcinoma. Oncotarget 2011;2:29–42.
[135] Gandellini P, Folini M, Longoni N, Pennati M, Binda M, Colecchia M, et al. miR-205 exerts tumor-suppressive functions in human prostate through down-regulation of protein kinase Cepsilon. Cancer Res 2009;69:2287–95.
[136] Friedman JM, Liang G, Liu CC, Wolff EM, Tsai YC, Ye W, et al. The putative tumor suppressor microRNA-101 modulates the cancer epigenome by repressing the polycomb group protein EZH2. Cancer Res 2009;69:2623–9.
[137] Ryan JM, Tivnan A, Bray I, Fay J, Davidoff AM, Tracey L, et al. MiR-204 acts as a tumor suppressor in neuroblastoma through down-regulation of the neurotrophic receptor TrkB. In: American Association for Cancer Research AACR 2011 – 102nd meeting; 2011.

[138] le Sage C, Nagel R, Egan DA, Schrier M, Mesman E, Mangiola A, et al. Regulation of the p27(Kip1) tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. EMBO J 2007;26:3699–708.
[139] Huang Q, Gumireddy K, Schrier M, le Sage C, Nagel R, Nair S, et al. The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. Nat Cell Biol 2008;10:202–10.
[140] Valastyan S, Reinhardt F, Benaich N, Calogrias D, Szász AM, Wang ZC, et al. A pleiotropically acting microRNA, miR-31, inhibits breast cancer metastasis. Cell 2009;137:1032–46.
[141] Björk JK, Sandqvist A, Elsing AN, Kotaja N, Sistonen L. miR-18, a member of Oncomir-1, targets heat shock transcription factor 2 in spermatogenesis. Development 2010;137:3177–84.
[142] Jiang S, Zhang HW, Lu MH, He XH, Li Y, Gu H, et al. MicroRNA-155 functions as an OncomiR in breast cancer by targeting the suppressor of cytokine signaling 1 gene. Cancer Res 2010;70:3119–27.
[143] Stenvang J, Kauppinen S. MicroRNAs as targets for antisense-based therapeutics. Expert Opin Biol Ther 2008;8:59–81.
[144] Brown BD, Naldini L. Exploiting and antagonizing microRNA regulation for therapeutic and experimental applications. Nat Rev Genet 2009;10:578–85.
[145] Czech MP. MicroRNAs as therapeutic targets. N Engl J Med 2006;354:1194–5.
[146] Wang Z. MicroRNA interference: an update. J Biol Med 2011;1:1–12.
[147] Torres AG, Fabani MM, Vigorito E, Gait MJ. MicroRNA fate upon targeting with anti-miRNA oligonucleotides as revealed by an improved Northern-blot-based method for miRNA detection. RNA 2011;17:933–43.
[148] Elmén J, Lindow M, Schütz S, Lawrence M, Petri A, Obad S, et al. LNA-mediated microRNA silencing in non-human primates. Nature 2008;452:896–900.
[149] Elmén J, Lindow M, Silahtaroglu A, Bak M, Christensen M, Lind-Thomsen A. Antagonism of microRNA-122 in mice by systemically administered LNA-antimiR leads to up-regulation of a large set of predicted target mRNAs in the liver. Nucleic Acids Res 2008;36:1153–62.
[150] Krützfeldt J, Kuwajima S, Braich R, Rajeev KG, Pena J, Tuschl T. Specificity, duplex degradation and subcellular localization of antagomirs. Nucleic Acids Res 2007;35:2885–92.
[151] Krützfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, et al. Silencing of microRNAs in vivo with 'antagomirs'. Nature 2005;438(7068): 685–9. ISSN 0028-0836.
[152] López-Fraga M, Wright N, Jiménez A. RNA interference-based therapeutics: new strategies to fight infectious disease. Infect Disord – Drug Targets 2008;8:262–73.
[153] Nielsen PE, Egholm M, Berg RH, Buchardt O. Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. Science 1991;254:1497–500.
[154] Demidov VV, Frank-Kamenetskii MD. Sequence-specific targeting of duplex DNA by peptide nucleic acids via triplex strand invasion. Methods 2001;23:108–22.
[155] Gambari R. Peptide-nucleic acids (PNAs): a tool for the development of gene expression modifiers. Curr Pharm Des 2001;7:1839–62.
[156] Paulasova P, Pellestor F. The peptide nucleic acids (PNAs): a new generation of probes for genetic and cytogenetic analyses. Ann Genet 2004;47:349–58.
[157] Karkare S, Bhatnagar D. Promising nucleic acid analogs and mimics: characteristic features and applications of PNA, LNA, and morpholino. Appl Microbiol Biotechnol 2006;71:575–86.
[158] Borgatti M, Lampronti I, Romanelli A, Pedone C, Saviano M, Bianchi N, et al. Transcription factor decoy molecules based on a peptide nucleic acid (PNA)-DNA chimera mimicking Sp1 binding sites. J Biol Chem 2003;278:7500–9.
[159] Nielsen PE. Antisense peptide nucleic acids. Curr Opin Mol Ther 2002;2: 282–7.
[160] Soomets U, Hällbrink M, Langel U. Antisense properties of peptide nucleic acids. Front Biosci 1999;4:D782–6.
[161] Ray A, Nordén B. Peptide nucleic acid (PNA): its medical and biotechnical applications and promise for the future. FASEB J 2000;14:1041–60.
[162] Nielsen PE. Targeting double stranded DNA with peptide nucleic acid (PNA). Curr Med Chem 2001;8:545–50.
[163] Good L, Nielsen PE. Progress in developing PNA as a gene-targeted drug. Antisense Nucleic Acid Drug Dev 1997;7:431–7.
[164] Marin VL, Roy S, Armitage BA. Recent advances in the development of peptide nucleic acid as a gene-targeted drug. Expert Opin Biol Ther 2004;4:337–48.
[165] Larsen HJ, Nielsen PE. Transcription-mediated binding of peptide nucleic acid (PNA) to double-stranded DNA: sequence-specific suicide transcription. Nucleic Acids Res 1996;24:458–63.
[166] Gambari R. Biological activity and delivery of peptide nucleic acids (PNA)-DNA chimeras for transcription factor decoy (TFD) pharmacotherapy. Curr Med Chem 2004;11:1253–63.
[167] Romanelli A, Pedone C, Saviano M, Bianchi N, Borgatti M, Mischiati C, et al. Molecular interactions with nuclear factor kappaB (NF-kappaB) transcription factors of a PNA-DNA chimera mimicking NF-kappaB binding sites. Eur J Biochem 2001;268:6066–75.
[168] Corradini R, Sforza S, Tedeschi T, Totsingan F, Marchelli R. Peptide nucleic acids with a structurally biased backbone: effects of conformational constraints and stereochemistry. Curr Top Med Chem 2007;7:681–94.
[169] Sforza S, Tedeschi T, Calabretta A, Corradini R, Camerin C, Tonelli R, et al. A peptide nucleic acid embedding a pseudopeptide nuclear localization sequence in the backbone behaves as a peptide mimic. Eur J Org Chem 2010;13:2441–4.
[170] Sforza S, Corradini R, Ghirardi S, Dossena A, Marchelli R. DNA binding of a D-lysine-based chiral PNA: direction control and mismatch recognition. Eur J Org Chem 2000;16:2905–13.
[171] Sforza S, Tedeschi T, Corradini R, Marchelli R. Induction of helical handedness and DNA binding properties of peptide nucleic acids (PNAs) with two stereogenic centres. Eur J Org Chem 2007;35:5879–85.
[172] Tedeschi T, Sforza S, Corradini R, Marchelli R. Synthesis of new chiral PNAs bearing a dipeptide-mimic monomer with two lysine-derived stereogenic centres. Tetrahedron Lett 2005;46:8395–9. ISSN 0040-4039.
[173] Dragulescu-Andrasi A, Zhou P, He G, Ly DH. Cell-permeable GPNA with appropriate backbone stereochemistry and spacing binds sequence-specifically to RNA. Chem Commun 2005;3:244–6.
[174] Fabani MM, Gait MJ. miR-122 targeting with LNA/2′-O-methyl oligonucleotide mixmers, peptide nucleic acids (PNA), and PNA-peptide conjugates. RNA 2008;14:336–46.
[175] Fabani MM, Abreu-Goodger C, Williams D, Lyons PA, Torres AG, Smith KGC, et al. Efficient inhibition of miR-155 function in vivo by peptide nucleic acids. Nucleic Acids Res 2010;38:4466–75.
[176] Abes R, Arzumanov A, Moulton H, Abes S, Ivanova G, Gait MJ, et al. Arginine-rich cell penetrating peptides: design, structure-activity, and applications to alter pre-mRNA splicing by steric-block oligonucleotides. J Pept Sci 2008;14:455–60.
[177] Borgatti M, Breda L, Cortesi R, Nastruzzi C, Romanelli A, Saviano M, et al. Cationic liposomes as delivery systems for double-stranded PNA-DNA chimeras exhibiting decoy activity against NF-kappaB transcription factors. Biochem Pharmacol 2002;64:609–16.
[178] Macadangdang B, Zhang N, Lund PE, Marple AH, Okabe M, Gottesman MM, et al. Inhibition of multidrug resistance by SV40 pseudovirion delivery of an antigene peptide nucleic acid (PNA) in cultured cells. PLoS One 2011;6(3):e17981.
[179] Zhou P, Dragulescu-Andrasi A, Bhattacharya B, O'Keefe H, Vatta P, Hyldig-Nielsen JJ, et al. Synthesis of cell-permeable peptide nucleic acids and characterization of their hybridization and uptake properties. Bioorg Med Chem Lett 2006;16:4931–5.
[180] Zhou P, Wang MM, Du L, Fisher GW, Waggoner A, Ly DH. Novel binding and efficient cellular uptake of guanidine-based peptide nucleic acids (GPNA). J Am Chem Soc 2003;125:6878–9.
[181] Nastruzzi C, Cortesi R, Esposito E, Gambari R, Borgatti M, Bianchi N, et al. Liposomes as carriers for DNA-PNA hybrids. J Control Release 2000;68: 237–49.
[182] Rasmussen FW, Bendifallah N, Zachar V, Shiraishi T, Fink T, Ebbesen P, et al. Evaluation of transfection protocols for unmodified and modified peptide nucleic acid (PNA) oligomers. Oligonucleotides 2006;16:43–57.
[183] Joergensen M, Agerholm-Larsen B, Nielsen PE, Gehl J. Efficiency of cellular delivery of antisense peptide nucleic acid by electroporation depends on charge and electroporation geometry. Oligonucleotides 2011;21:29–37.
[184] Shiraishi T, Nielsen PE. Enhanced cellular delivery of cell-penetrating peptide-peptide nucleic acid conjugates by photochemical internalization. Methods Mol Biol 2011;683:391–7.
[185] Ishihara T, Kano A, Obara K, Saito M, Chen X, Park TG, et al. Nuclear localization and antisense effect of PNA internalized by ASGP-R-mediated endocytosis with protein/DNA conjugates. J Control Release 2010. doi: 10.1016/j.conrel.2010.10.014.
[186] Oh SY, Ju YS, Park H. A highly effective and long-lasting inhibition of miRNA with PNA-based antisense oligonucleotides. Mol Cells 2009;28:341–5.
[187] Fabbri E, Bianchi N, Brognara E, Finotti A, Breveglieri G, Borgatti M, et al. Inhibition of micro RNA 210 biological activity with an anti-miR-210 peptide nucleic acid. Int J Mol Med 2010;26:S61.
[188] Marchelli R, Corradini R, Manicardi A, Sforza S, Tedeschi T, Fabbri E, et al. Gene modulation by peptide nucleic acids (PNAs) targeting microRNAs (miRs). ISBN: 978-953-307-540-2, InTech, Available from: http://www.intechopen.com/articles/show/title/gene-modulation-by-peptide-nucleic-acids-pnas-targeting-micrornas-mirs-.
[189] Yan LX, Wu QN, Zhang Y, Li YY, Liao DZ, Hou JH, et al. Knockdown of miR-21 in human breast cancer cell lines inhibits proliferation, in vitro migration and in vivo tumor growth. Breast Cancer Res 2011;13:R2. doi: 10.1186/bcr2803.
[190] Wahid F, Shehzad A, Khan T, Kim YY. MicroRNAs: synthesis, mechanism, function, and recent clinical trials. Biochim Biophys Acta 2010;1803: 1231–43.
[191] Mack GS. MicroRNA gets down to business. Nat Biotechnol 2007;25:631–8.