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Overview

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Abstract

RNA interference (RNAi) is an epigenetic mechanism involved in regulation of gene expression. It relies on small non-coding RNAs: endogenous miRNA and exogenous siRNA. Among various vital processes in the cell, RNAi regulates the cell cycle progression and apoptosis. Hence, (epi)mutations of the RNAi system can force the cell toward promotion of proliferation and evasion of apoptosis leading to neoplastic transformation. Aberrant RNAi is commonly found in various cancers or even precancerous lesions and seems to be involved in anti-cancer drug resistance as well. Therefore biomedical science nowadays tends to identify RNAi profiles and translate them into clinical biomarkers for cancer diagnostics and therapy response. Since exogenous branch of RNAi pathway sustains specific iatrogenic regulation of gene expression, intense attempts of developing novel anticancer therapy approaches using synthetic small non-coding RNAs are on-going. Several experimental RNAi therapies in different stages of clinical trials are expected to inhibit cell proliferation and re-establish apoptotic activity in transformed cells. In this review we focus on recent discoveries of RNAi regulating apoptosis, enclose these findings in cancer biology context and discuss potential translation of the field into clinical medical practice considering novel trends in management of cancer.

INTRODUCTION

Human genome project granted the modern science with an unexpected finding that human genome has only about 30,000 protein coding regions, leaving almost 98% of the sequence simply non-coding. With the idea to identify and map this predominant element of our genome, National Human Genome Research Institute (NHGR) was encouraged to launch a whole new project named Encyclopaedia of DNA Elements (ENCODE) *(1)*. Besides elucidating the characteristics of non-coding regions, ENCODE's main goal is to reveal their potential function and place in various biological processes within the cell. Major progress in technology and scientific research in this new biological field is certainly expected to provide further insights in development of various diseases as well as shape an entirely new platform for future medicine. Recently ENCODE revealed that 80% of human genome is indeed non-coding but somehow biochemically functional *(2)*. Among the transcription products of those non-coding regions is a group of small non-coding RNAs which are not translated but rather involved in other mechanisms vital to the cell and therefore the individual as a whole. Concisely, small RNAs act as a defensive mechanism against parasitic nucleic acids but more importantly as posttranscriptional regulators of gene expression through the mechanism called RNA interference

(RNAi) *(3-4)*. The ability to regulate gene expression without changing the DNA sequence makes RNAi a powerful epigenetic mechanism. In fact, RNAi is nowadays accepted as one of the three most prominent epigenetic maintenance mechanisms shaping a very core of epigenetics alongside with DNA methylation and histone modifications *(5)*. The phenomenon of RNA interference (RNAi) was first found in the nematode *Caenorhabditis elegans* after introduction of a double-stranded RNA which led to specific gene silencing *(6)*. Only eight years after mentioned findings, the importance of RNAi was acknowledged with Nobel Prize in 2006. Namely, not long after the first discovery it became apparent that RNAi is present in almost every organism from plants to mammals highlighting RNAi as a vital cellular pathway in most if not all eukaryotes *(7-8)*.

RNAi PATHWAY

Carriers of RNAi are small RNAs, from 21 to 23 nucleotides in size, and can be classified as microRNA (miRNA) or small-interfering RNA (siRNA) according to the origin of the molecule, endogenous or exogenous, respectively (Fig. 1.). In the nucleus, primary-microRNA (pri-miRNA) is transcribed *(9)* and processed into a hairpin precursor pre-miRNA by an enzyme RNase III or DROSHA *(10-11)*. Pre-miRNA binds Exportin 5 which is a RanGTP-dependent dsRNA-binding protein. By this binding Exportin 5 mediates pre-miRNA export to the cytoplasm *(12)*. In the cytoplasm pre-miRNA is cleaved by DICER into the mature miRNA duplex *(13-14)* which is loaded on Argonaute (AGO) protein *(15)* forming precursor of RNAi-induced silencing complex (pre-RISC) *(16)*. One strand of the miRNA duplex, called the passenger strand, is removed and the other, called the leading strand, guides silencing complex to target mRNA for posttranscriptional silencing. SiRNA follows the same pathway but joins it in the later step rather than in the nucleus as miRNA. Namely, siRNA originates from outside of the cell and therefore begins the RNAi pathway after entering into the cytoplasm through the cell membrane. In the cytoplasm, long double stranded siRNA precursors are processed by DICER into the double stranded siRNA similar as pre-miRNA. In the pre-RISC complex AGO cleaves the passenger strand while the mature RISC complex is guided by the leading siRNA strand to the complementary target mRNA for silencing. Although sharing the same cellular pathway and inducing similar epigenetic effect, miRNA and siRNA show important difference in specificity. Namely, miRNA is more promiscuous regarding the target since one miRNA can recognize multiple targets. In fact, miRNA recognizes only a small fragment of target mRNA, called the "seed" region, constructed of up to eight but possibly down to

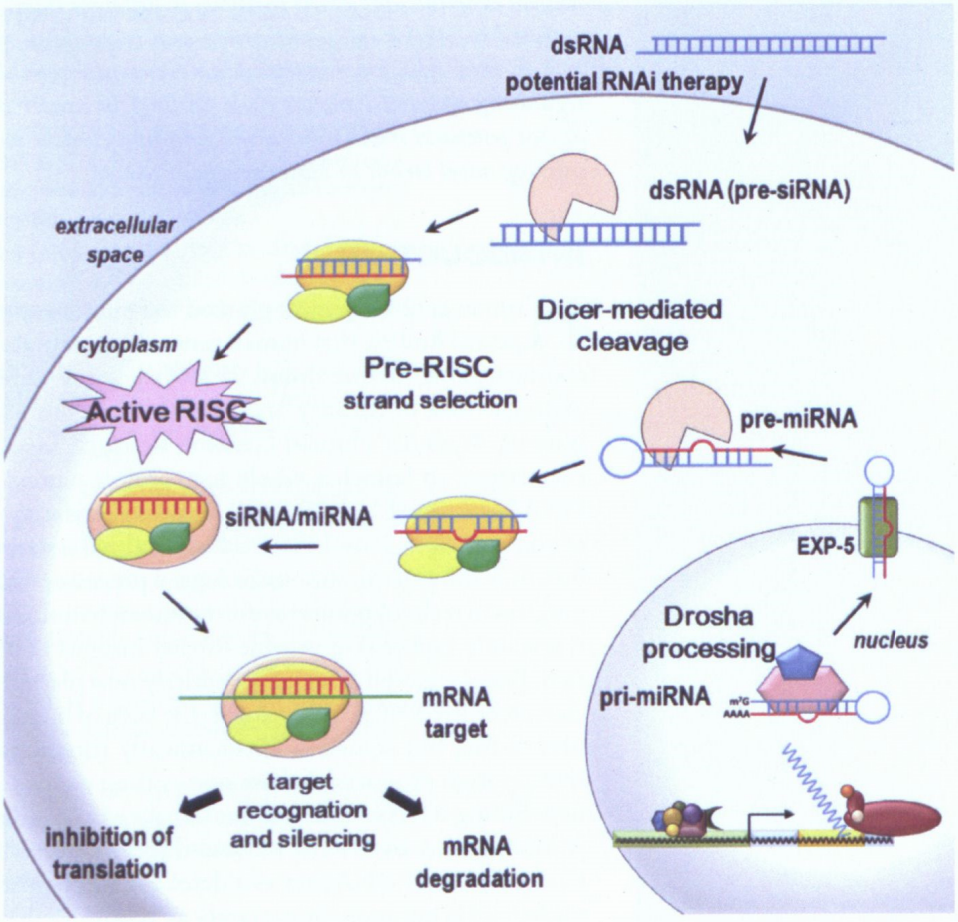

Figure 1. *RNAi pathway.*

only two nucleotides. On the contrary, siRNA recognizes a longer fragment of mRNA and is usually fully complementary to its target region *(17-18)*. Such a perfect match results with target mRNA degradation while a partial one induces translational repression *(19)*. Therefore, the level of complementarity defines the outcome of silencing.

Small RNAs control various cellular pathways under physiological conditions by regulating gene expression of e.g. tumour suppressors and oncogenes, cell cycle genes and DNA repair machinery genes *(20)*. Therefore, deregulation of small RNAs expression is considered as epigenetic mutation (epimutation) that can initiate and promote serious changes in cell homeostasis through changes of proliferation, differentiation or apoptosis leading to cancer and cancer related events. Evidences of RNAi involvement in cancer biology led to further affirmation of epigenetics because it became obvious that neoplastic transformation can be caused by much more than gene mutations or chromosome aberrations. More important, discovery and further understanding of RNAi provided a new focus in cancer research with the establishment of new diagnostic and prognostic biomarkers as well as development of new potential therapeutic approaches. Indeed, only one decade after the first discovery and although still facing numerous setbacks, RNAi based therapies entered clinical trials. Therefore, in this review we shall focus on recent discoveries in RNAi regulating apoptosis and proliferation, enclose these findings in cancer biology context and discuss potential translation of the field into medical practice considering cancer management.

MODULATION OF APOPTOSIS BY SMALL RNAS IN CANCER

Since small RNAs are involved in cell cycle regulation their role in cancerogenesis was correctly foreseen. Today, new terms are rising for miRNAs engaged in cancerogenesis, oncomirs for cancer related miRNAs, apoptomirs for apoptosis related and even metastamirs for miRNAs involved in metastasis of cancer cells *(21)*. In fact, Kumar demonstrated global repression of miRNA processing machinery to promote neoplastic transformation, cancer formation and progression *(22)*. Indicatively, microRNA coding regions seem to be located at cancer-associated genomic regions or in fragile sites. Various mutations and epimutations in RNAi machinery have been proven to initiate or promote deregulation of the cell cycle as well as of the apoptosis (Fig. 2.). This critical event in neoplastic transformation provides to the unstable, genetically altered and damaged cells the advantage of relentless cell cycle resulting in further cancer growth and advancement of neoplastic development.

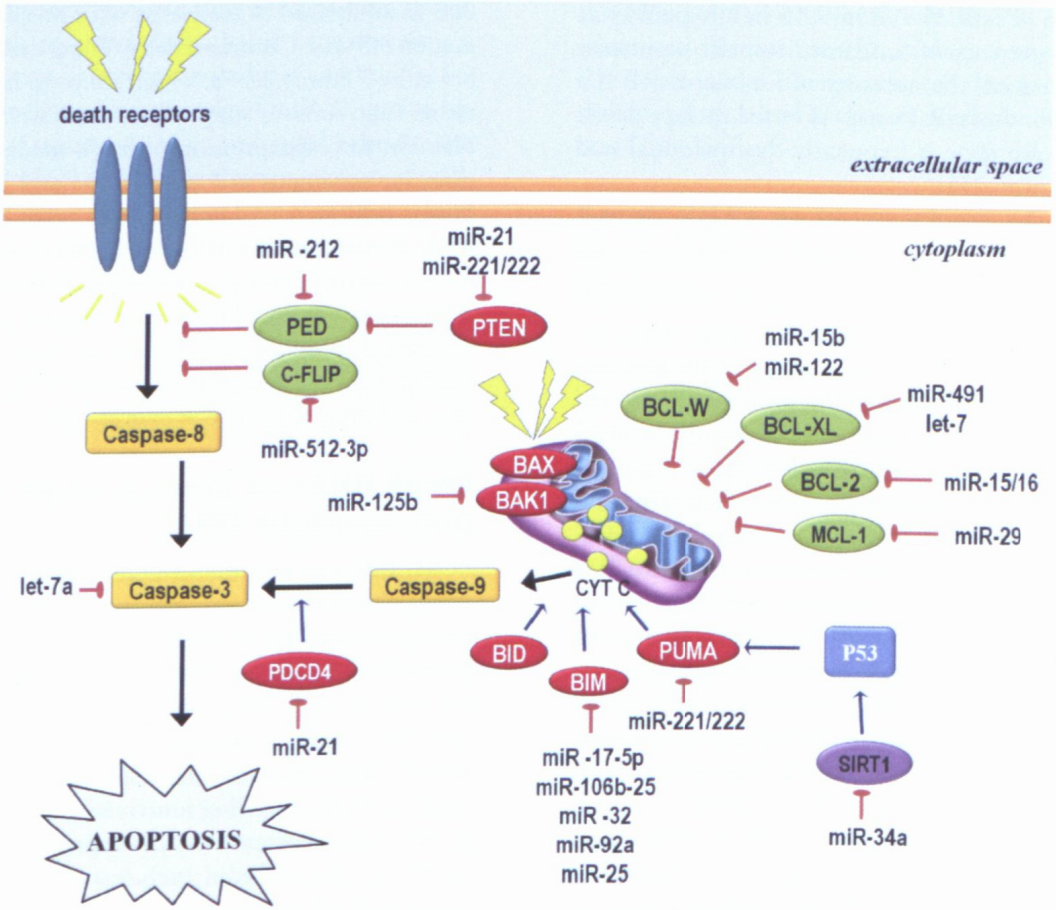

Figure 2. *MiRNAs regulating apoptotic pathways.*

Small RNAs with tumour suppressor activity: pro-apoptotic RNAi

BCL-2 is a known proto-oncogene first discovered in follicular B cell lymphoma cells carrying chromosome translocation (14;18) *(23-24)*. It is an anti-apoptotic protein that resides on the outer mitochondrial membrane preventing release of the Cytochrome C (CYT C) and activation of the intrinsic apoptotic pathway. Because of its role in inhibition of apoptosis it is often overexpressed in human malignancies such as breast cancer *(25)*, Hodgkin's lymphoma *(26)* and B-cell lymphoma *(27)*. Cimmino showed that levels of expression of BCL-2 inversely correlated with miR-15 and miR-16 in cancer cells. They also described a potential miR15-a and miR16-1 binding site at 3'UTR region of BCL-2 mRNA (28). This was one of the first discoveries highlighting the potency of miRNA in regulation of apoptosis. MiR-15 and miR-16 form a cluster at the chromosome 13q14, region deleted in many cancers including mantle cell lymphoma *(29)*, multiple myeloma *(30)*, breast cancer *(31)* and prostate cancer *(32)*. Moreover, miR-15 and miR-16 were found to be downregulated in 68% of chronic lymphocytic leukaemia (CLL) *(33)*. MiR-15 and miR-16 are also downregulated in prostatic cancer where they target proliferation and survival promoting factors CCND1 and WNT3a (34). In non–small cell lung cancer (NSCLC) miR-15 and miR-16 negatively regulate expression of Cyclin D1, Cyclin D2 and Cyclin E1. It is important to emphasize that overexpression of miR-15 and miR-16 induces cell cycle arrest only in presence of functional tumour suppressor RB protein. Indeed, the necessity of functional RB is a huge setback for the miR-15 and -16 based therapy development since Rb gene is frequently dysfunctional and changed in cancers *(35)*.

Tumour suppressive activity of RNAi relies on miR-34 family as well. Although numbering three miRNAs, miR-34 family is coded by only two genes, miR-34a and miR-34b/34c, respectively. MiR-34 gene is a direct transcriptional target of P53, the guardian angel of the genome. As is well known, P53 is one of the most important and most studied tumour suppressors which mediate cell response to DNA damage, nutrient deprivation, hypoxia and any other exogenous or endogenous stress. Depending on the level of damage, it regulates the cell cycle progression and induces apoptosis. In context of RNAi, P53 seems to have the ability to activate miR-34 promoter resulting in integration of miR-34 into apoptotic pathways. Chang tested this hypothesis showing pancreatic cancer cell lines to possess a remarkable decrease in miR-34 levels compared to control pancreatic ductal epithelial cells. Therefore, Chang postulated that expression of miR-34 induces reprogramming of gene expression which regulates cell cycle and promotes apoptosis *(36)*. Apart from p53 dependent miR-34 activation, during oncogene–induced senescence, miR-34 is activated in p53-independent manner. In this scenario, miR-34 gene is transcriptionally regulated by the ELK1 transcription factor. Through this pathway, miR-34 seems to inhibit c-Myc and contribute to the cell cycle arrest as well as senescence *(37)*. MiR-34 independency of p53 was confirmed by Jis experiment of restored miR-34 expression in p53 mutant gastric cancer cell lines. Renewed miR-34 expression resulted in decrease of BCL-2, HMGA2 and NOTCH followed by inhibition of growth and tumoursphere formation *(38)*. In addition, miR-34 inhibition was proven to enhance expression of well-known proto-oncogenes like BCL2, CDK6, MET and CCND (37). MiR-34 was found to be downregulated in NSCLC as well *(39)*. Moreover, decreased expression of miR-34 was found in 36% of colon cancers compared to normal tissue while enforced expression of miR-34 in colon cancer cell lines suppressed E2F family of transcription factors and inhibited proliferation emphasizing tumour suppressive role of miR-34 (40).

Let-7 microRNA precursor family was identified from a study of developmental timing in *C. elegans (41)* but soon after associated with RNAi machinery in cancer biology *(42)*. Let-7 microRNAs are downregulated or lost in various cancers indicating their role as tumour suppressors *(43)*. Let-7 cluster is downregulated in human lung cancer and it was even associated with worse prognosis independent of the stage of disease *(44)*. Another target of let-7 family is the HMGA2 gene which is active during embryonic development while its inappropriate expression in adulthood is associated with neoplastic transformation *(45-46)*. Controversially, Tsang described a member of let-7 family, let-7a, which seems to have oncogenic rather than tumour suppressive function in cancer cells. Namely, forced expression of let-7a made doxorubicin-resistant squamous cell cancer cells and hepatocellular cancer cells even more resistant to several cancer drugs while downregulation of let-7a resulted in acquiring apoptosis-sensitive phenotype. Moreover, low let-7a pro-apoptotic phenotype could be further reversed by introducing Caspase-3 inhibitor *(47)*. Therefore, it seems that let-7a inhibits Caspase-3 activity thus promoting apoptosis evasion and drug resistance of cancer cells.

Small RNAs as proto-oncogenes: anti-apoptotic RNAi

MiR-21 was first found upregulated in glioblastoma where it functions as an oncogene by suppressing apoptotic pathway *(48)*. Its overexpression is found in diverse type of malignancies such as breast cancer *(49)*, pancreatic cancer *(50)*, oesophageal cancer *(51)*, NSCLC *(52)* and colon adenocarcinoma where it was associated with poor survival and poor therapeutic outcome *(53)*. MiR-21 has a survival promoting function because it targets some of the most important tumour suppressors in the cell such as PTEN. Meng found excessive expression of miR-21 using miRNA microarray technology in hepatocellular cancer (HCC). Overexpression of miR-21 in HCC cells

downregulated PTEN and several PTEN-dependent pathways which enhanced cell proliferation, migration and invasion. This was confirmed by transfecting normal human hepatocytes with miR-21 precursors leading to its overexpression resulting in increased migration of cells *(54)*. Although miR-21 overexpression was found to be oncogenic in healthy liver, after tissue injury the same overexpression was found to promote liver regeneration by again promoting cell cycle and inhibiting apoptosis *(55)*. Furthermore, miR-21 enhances cell invasion and migration by interacting with PDCD4 mRNA as well *(56)*. PDCD4 is an inhibitor of neoplastic transformation which suppresses activation of AP-1-dependent transcription *(57)*. It was proven that indeed miR-21 negatively regulates PDCD4 in oesophageal squamous cell cancer cells *(58)* and breast cancer cells (59) giving to the cells growth advantage and facilitating malignant transformation. Recent study on a MCF-7 breast cancer cell model showed that after introducing miR-21, cells started expressing mesenchyme cell markers while at the same time inhibiting epithelial markers expression. It was a clear sign of miR-21 involvement in epithelial-mesenchymal transition, already described as the critical moment for tumour progression and metastasis *(60)*. MiR-21 is also indirectly involved in regulation of p53 and TGF-β pathway in glioblastoma cells. It targets p53 homologues p63 and p73 as well as p53 activators JMY, TOPORS, TP53BP2, DAXX, and HNRPK which are essential for functional P53 tumour-suppressive activity *(61)*. Interestingly, epimutation of miR-21 with resulting deregulation of apoptosis was also found in non-cancerous pathologic conditions. For example, Gabriely studied the role of miR-21 during hypoxia in cardiac myocytes. After prolonged hypoxic conditions, they noticed significant downregulation of miR-21 resulting in higher expression of PTEN and FasL, both of which mediate the main apoptotic pathway in heart failure and ischemia *(62-64)*.

Apart from miR-21, numerous miRNAs were associated with various cancer initiation and progression processes. MiR-221 and 222 are upregulated in human thyroid papillary cancer (PTC) in which they target tumour suppressive $p27^{Kip}$, cell-cycle regulatory protein *(65-66)*. MiR-221/222 was also overexpressed in aggressive prostatic cancer cells (PC3) compared to less aggressive cancer which indicates its potential role in cancer progression and ranking of malignant phenotype *(67)*. Involvement in cancer progression and invasion was also noted in hepatocellular and lung cancers *(68)*. Further investigations led to Garofalos discovery that miR-221/222 interfere with TRAIL-dependent apoptosis pathway. Namely, their suppression with antisense RNAs in TRAIL resistant NSCLC cells makes the cells TRAIL sensitive, presumably by upregulation of p27. Regardless of the exact mechanism, it is believed that introduction of their inhibitors into drug resistant cancer cells should impel apoptotic machinery and therefore sensitize cancer cells to therapy *(69)*. Indeed, it was already described that in human bladder cancer suppression of miR-221 induces TRAIL-mediated apoptosis *(70)*. In addition, miR-221/222 target mRNA of pro-apoptotic protein PUMA in glioma and their knock-down resulted in apoptosis and arrest in tumour growth *(71)*. MiR-221/222 can be suppressed by promyelocytic leukaemia zinc finger (PLZF) transcription factor in melanoma which inhibits proliferation and malignant progression (72). Interestingly, He et al. found negative correlation between miR-221/222 and c-Kit, oncogenic tyrosine kinase which is extremely low in thyroid cancer *(73)*. This is noteworthy since c-kit is usually regarded as an oncogene but in the case of thyroid cells it is probably involved in differentiation rather than transformation of the epithelial tissue *(74)*. In several patients miR-221/222 were expressed even in healthy tissue around papillary thyroid cancer suggesting their role in cancer initiation *(75)*. This finding is in concordance with broadly accepted hypothesis that histopathologically recognizable precancerous lesions i.e. cell phenotypes are preceded with histologically undetectable deregulation in epigenome *(76)*.

MiR-29 family manifests its tumour suppressive pro-apoptotic activity again through upregulation of P53 and suppression of regulatory subunit of PI3 kinase and CDC42 *(77)*. MiR-29b targets Mcl-1 so it is found downregulated in cholangiocarcinoma *(78)*. Elevated expression of Mcl-1 causes TRAIL- resistant phenotype through inhibition of Cytochrome C release *(79)*. In addition to what was discussed in this review, a number of small RNAs are under intense research due to their foreseen task in cell cycle and apoptosis regulation in various cancers (Tab. 1).

Small RNAs as biomarkers of the response to conventional cancer therapy

Due to their genetic instability, neoplastic cells often find a way to escape apoptosis induced by chemotherapy. Insensitivity to conventional anti-cancer drugs or acquirement of drug resistance during treatment is one of the main clinical problems in treating cancer. Mechanism of acquiring this resistance is still mainly unclear, but since most chemotherapeutics are cytotoxic and tend to induce apoptosis it is reasonable to expect that RNAi, as an apoptotic modulator, is somehow involved in modulating or even establishing anti-cancer drug resistance. Verily, miRNAs were recently confirmed to be involved in this aspect of cancer and every day additional data, showing that aberrant levels of small RNA can modulate drug response, are presented. Indeed, in human breast cancer, reduced levels of miR-375 have been found in tamoxifen-resistant breast cancer cells and miR-298 in doxorubicin-resistant human breast cancer *(78-79)*. Conversely, miR-21 was shown to mediate breast cancer resistance to trastuzumab, miR-34a to docetaxel and miR-221/222 to

TABLE 1

Small RNAs targeting most prominent segments of apoptotic pathways in various cancers.

	Small RNA	Target	Cancer	Reference
Pro-apoptotic	***miR*-224**	API-5	hepatocellular cancer	*(110)*
	miR-15/16	BCL-2	chronic myeloid leukaemia	*(28)*
	***miR*-122**	BCL-W	hepatocellular carcinoma	*(111)*
	***miR*-15b**	BCL-W	hepatocellular carcinoma	*(112)*
	miR-491	BCL-XL	colorectal cancer	*(113)*
	let-7 (c,g)	BCL-XL	hepatocellular carcinoma	*(114)*
	***miR*-212**	PED	non-small cell lung cancer	*(115)*
	***miR*-512-3p**	c-FLIP	hepatocellular carcinoma	*(116)*
Anti-apoptotic	***miR-125b***	BAK1	prostate cancer	*(117)*
	miR-17-5p	BIM	neuroblastoma	*(118)*
	miR-106b-25	BIM	oesophageal adenocarcinoma	*(119)*
	miR-32	BIM	prostate cancer	*(120)*
	miR-92a	BIM	colon cancer	*(121)*
	miR-25	BIM	ovarian cancer	*(122)*
	miR-186	Caspase-10	lung cancer	*(123)*
	miR-24	FAF1	prostate cancer	*(124)*
	miR-21	PTEN	hepatocellular cancer	*(54)*

fulvestrant and tamoxifen. The knock down of those miRNAs, as a pre-treatment, made cells sensitive to drug induced apoptosis and cell cycle arrest *(1, 80-82)*. Zhou suggested that miR-125b targets pro-apoptotic BAK1 in breast cancer what leads to paclitaxel induced apoptosis resistance *(83)*. Therefore miR-125b circulating levels were a reliable indicator of chemoresistance (84). MiR-125b also promotes cisplatin chemoresistance in ovarian cancer through the same mechanism *(85)*. MiR-130a modulates cisplatin treatment response in ovarian cancer as well but its overexpression was associated with ABC drug transporter superfamily and cell survival promoting PI3K/Akt pathway *(86)*. Kojimas group found that miR-34a was reduced in paclitaxel resistant prostate cancer cells with consequential upregulation of SIRT1 and BCL-2 *(87)*. In advanced stages of prostate cancer, miR-205 and miR31 which target anti-apoptotic BCL-W and E2F6 were suggested to protect from developing chemotherapy resistance *(88)*. In colon cancer miR-222 targets ADAM 17, a protein showed to be responsible for developing multidrug resistance. Interestingly, in the same study authors inhibited ADAM-17 with siRNA or the small molecule ADAM-17 inhibitor TAPI-2 and combined it with chemotherapy which resulted in resensitizing multidrug resistant cells for therapy *(89)*. According to Xia's discovery, miR-15b and miR-16 are downregulated in multidrug-resistant human gastric cancer cell line. After transfection with miR-15b or miR-16 precursors, cancer cells showed improved sensitivity to vincristine, adriamycin, cisplatin and etoposide, probably through modulating BCL-2 mediated apoptotic pathways *(90)*. In gastric cancer both miR-15, miR-16 and miR-181b were reduced in vincristine resistant cancer cell lines followed by upregulation of BCL-2 *(90-91)* as well as miR221 and miR-222 which regulate radioresistance by targeting PTEN *(92)*. In lung cancer, miR-34c was suggested to protect cancer cells from paclitaxel induced apoptosis targeting pro-apoptotic BCL-2 family member BMF *(93)*. In hepatocellular cancer miR-214 was significantly up-regulated in gefitinib resistant cell line followed with underexpression of PTEN. By knocking-down miR-214, resistant phenotype was reversed *(94)*. Hence, since aberrant miRNAs signature seems indeed to be associated with drug responsiveness, screening for specific miRNAs as biomarkers could discriminate patients receptive to a specific anticancer therapy before starting a treatment.

Small RNA as novel cancer therapy: from basics to clinics

The potential therapeutic value of RNAi is strongly supported by oncogene addiction phenomenon. This novel concept postulates that, even though cancer is characterised as a polygenic disorder, its survival and progression is driven by just one or few genes and their inhibition

should lead to cancerous cell death *(95)*. Since, oncomirs are regarded as "novel oncogenes" it gives us a whole new ground to develop a specific oncomir-targeted therapy. This hypothesis was confirmed by Medina who induced pre-B-cell lymphoma by reversible knocking-in miR-21 *in vivo*. Subsequent knocking-down induced complete tumour regression in a few days partially through apoptosis *(96)*. In addition, the intent of translating RNAi into clinical practice stimulates development of many scientific fields and technology. Namely, apart from elucidating numerous, still obscure impacts on the cell, before getting benefits from RNAi in medical practice there are practical barriers that need to be overcome. Possibly one of the most prominent obstacles is *in vivo* delivery of small RNAs to the target cell and tissue. In cell culture experiments delivery does not represent any problem since there are plenty of elaborated delivering systems available like transfection with lipofectamine. Biologically, RNAs are relatively sensitive molecules which, after introduction to the human or animal body, are easily digested by serum nucleases and excreted through kidneys. Therefore it is difficult to obtain a proper therapeutic concentration of the small RNA in the body. Those RNAs that survived degradation and excretion can onset unwanted IFN-1 mediated immune response and activate immune system by interacting with Toll-like receptors *(97-98)*. Even if reaching the cell, small RNA molecule has still a relatively big molecular weight and negative charge what makes it difficult to penetrate through the cell membrane. Most, if not all mentioned problems may be avoided by chemical modifications of small RNA molecule or by conjugating it with some substance which makes it more stable *in vivo*. For instance, 2β-***O***-methyl modification of siRNA backbone makes small RNA resistant to serum nucleases, decreases immunogenicity and lowers off-targeting *(99-102)*. Apart from developing breaking through therapeutic small RNA complexes, searching for optimal dose is the second important parameter that needs to be addressed in order to ensure a safe and beneficial therapeutic result. Namely, one of the first attempts of *in vivo* gene silencing by RNAi on a murine model led to dose dependent toxicity. Grimm introduced high levels of hairpin miRNA precursors which led to saturation of RNAi machinery and toxic accumulation of endogenous miRNAs in the nucleus *(103)*. RNAi toxicity could be dodged by the use of siRNA instead of hairpin precursor since it enters RNAi pathway several steps later, sidestepping saturation of Exportin 5 proteins. Another important issue regarding small RNA is off-target silencing via non-specific pairing of just few nucleotides that can silence normally functioning genes and potentially give serious side effects *(104)*. Even though, knowing the sequence of the Human genome enables the industry for *in vitro* synthesis of RNAi active polynucleotides with great specificity, low side-effect risk and at relatively low cost. So far, there is still no RNAi based therapy approved, but there are numerous ongoing research projects as well as several clinical trials (Table 2.). Among RNAi cancer therapies in clinical trials most are using non-viral delivery methods to target oncogene driving cancer progression to induce cell cycle arrest and apoptosis of cancerous cells. For example, ALN-VSP (Alnylam) is SNALP (stable nucleotide acid lipid particle) based technology which targets VEGF and KSP, two proteins required for tumour angiogenesis and cell proliferation. Results of phase I clinical trial demonstrated that most patients with advanced metastatic

TABLE 2

RNAi based therapies under clinical trials.

Drug	Target	Cancer	Trial phase	Sponsor	Clinical trial ID
ATU-027	PKN3	advanced and metastatic solid tumours	Phase I completed	Silence Therapeutics	NCT00938574
ALN-VSP02	VEGF, KSP	solid tumours	Phase I completed	Alnylam	NCT00882180
TKM-PLK1	PLK1	liver tumour	Phase I recruiting	Tekmira	NCT01262235
CALAA-01	RRM2	solid tumour	Phase I ongoing	Colando Pharmaceutics	NCT00689065
FANG	Furin	solid tumours	Phase I recruiting	Gradalis Inc.	NCT01061840
siG12D LODER	KRASG12D	pancreatic tumour	Phase I recruiting	Silenseed Ltd.	NCT01188785
CEQ508	β-catenin	FAP	Phase I recruiting	Marina Biotech	/
siRNA/mRNA transfected dendritic cells	LMP2, LMP7, MECL1	metastatic melanoma		Duke university	NCT00672542

disease experienced disease stabilization and showed satisfying tolerance to therapy. Tekmira is developing another therapy approach which targets kinase PLK1. Various cancers have elevated levels of PLK1 what promotes cancer cell proliferation and avoidance of apoptosis *(105-106)*. Miniature biodegradable polymer siG12D LODER (Silenseed) is carrying siRNA against mutated form of KRAS gene, frequently expressed in pancreatic cancers *(107-108)*. And finally, Marina Biotech is developing therapy for familial adenomatous polyposis coli (FAP), named CEQ508. CEQ508 is small RNA against β-catenine in an attenuated *Escherichia coli* vector which induces cleavage of oncogene mRNA and leads to death of the targeted cancerous cells *(109)*.

CONCLUSION

Although a recently discovered biological phenomenon, RNAi has already been well incorporated in various fields of biomedical basic research, especially cancer biology. Indeed, according to basic studies, implementation of RNAi into medical practice seems feasible and highly promising. As discussed in this review, RNAi deregulations and epimutations are commonly found in various cancers and believed to be involved in cancer initiation, progression and metastatic potential. Consequently, RNAi has already been recognized as potential signature of cancers and their subtypes as well as an indicator of therapy response and prognosis in patients. Apart from potential use as a biomarker in diagnostics, RNAi based therapy is already experiencing translation into medical practice relying on downregulation of oncogenes expression and reestablishment of apoptosis. Noteworthy, RNAi based drugs seem to be highly specific, well tolerated and cheap to produce. Undefeated barrier of delivering therapeutic RNAs to the target tissue in the living organism still remains.

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