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Review

The role of microRNAs in cancer: No small matter

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ABSTRACT

MicroRNAs are a recently discovered class of small, evolutionarily conserved, RNA molecules that negatively regulate gene expression at the post-transcriptional level. Mature microRNAs of approximately 20–22 nucleotides are formed from longer primary transcripts by two sequential processing steps mediated by a nuclear (Drosha) and a cytoplasmic (Dicer) RNAse III endonuclease. In the context of a protein complex, the RNA-induced silencing complex (RISC), microRNAs base-pair with target messenger RNA sequences causing translational repression and/or messenger RNA degradation. MicroRNAs have been implicated in the control of many fundamental cellular and physiological processes such as tissue development, cellular differentiation and proliferation, metabolic and signalling pathways, apoptosis and stem cell maintenance. Mounting evidence indicates that microRNAs also play a significant role in cellular transformation and carcinogenesis acting either as oncogenes or tumour suppressors. This review briefly introduces microRNAs in a historical perspective and focusses on the biogenesis of microRNAs, their mode of action, mammalian microRNA functions with emphasis on their involvement in disease – particularly cancer – and their potential therapeutic use.

1. Non-protein coding RNAs

The transcriptional output of the mammalian genome is impressive as at least 65% of the genome is transcribed on one or both strands.[1,2] However, all known and predicted protein coding transcripts are together derived from less than 2% of the human genome. Systematic sequencing of full-length cDNA libraries[3,4] and the examination of the transcriptome by genome tiling studies[5–7] reveal that non-protein coding transcripts (npcRNAs) are abundant and may account in mammals for at least half of all transcripts present. Apparently, the mammalian genome harbours many genes that encode npcRNAs that in a growing number of instances do seem to be genetically active, have important regulatory roles in normal cell physiology and can be dysregulated in disease[8] (see also http://research.imb.uq.edu.au/RNAdb). In particular, the recent discovery of a rather well-defined group of small npcRNAs: the microRNAs (miRNAs) and their involvement in many elementary cellular processes suggest that we are only just beginning to understand the complexity and functional impact of miRNAs and other npcRNAs.[9]

2. A newly recognised class of ribo-regulators: microRNAs

MiRNAs are small 16–29 nucleotide RNA molecules that comprise an evolutionarily conserved class of endogenous ribo-regulators that modulate gene expression via the RNA interference (RNAi) pathway. RNAi is a post-transcriptional silencing mechanism, present in most eukaryotic organisms, in which exposure to double-stranded RNA (ds-RNA) induces

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the sequence specific degradation of homologous messenger RNAs (mRNAs).[10] The mediators of RNAi are the short interfering RNAs (siRNAs), small npcRNAs of ~22 nucleotides that are processed from the exogeneously introduced ds-RNA. The RNAi pathway, that functions in cellular defence protecting the cells against, e.g. viral infections, has been widely exploited as a silencing tool to disrupt the activity of cellular genes. Like siRNAs endogenous miRNAs act by base-pairing, in the context of an RNA-induced silencing complex (RISC), with their target mRNAs. In contrast to siRNAs that usually display a perfect complementarity to their target sequences and trigger target mRNA degradation, miRNAs generally exhibit only a partial complementarity and often bind in 3′ untranslated region (3′ UTR) of target mRNAs leading to translational repression and/or degradation (see for reviews[11–13]).

Already in 1993 miRNAs were discovered in the worm *Caenorhabditis elegans* by Ambros and co-workers using forward genetics. By characterising *C. elegans* mutants disturbed in normal development, they discovered that a 22 nucleotide transcript of *lin-4* modulated developmental timing by acting as a negative regulator of the protein coding gene *lin-14*.[14,15] It was noted that *lin-4* displayed a partial sequence complementarity to a sequence repeat in the 3′ UTR of *lin-14* leading the authors to postulate that *lin-4* operates by interacting with these 3′ UTR elements inhibiting in this way the translation of *lin-14*.[14] Initially, miRNAs were considered a peculiarity of nematodes until it was found that the sequence and temporal expression pattern of another miRNA involved in developmental timing in *C. elegans let-7*[16] was phylogenetically conserved in a wide variety of animals including humans.[17] This observation prompted the construction and characterisation of several large-scale cDNA libraries enriched for small RNAs and led to the identification of many additional miRNAs from plants, *C. elegans*, *Drosophila* and mammals.[18–24] Many miRNAs have a tissue-specific expression, suggesting that they fulfil roles in tissue specialisation and/or tissue maintenance.[19,20,25] Currently, 4167 miRNAs are listed in the miRNA registry, version 9[26] (http://microrna.sanger.ac.uk) derived from a variety of organisms like plants, viruses, arthropods and vertebrates such as amphibians, birds, fish and mammals. To date, 474 human miRNAs have been identified either through random cloning and sequencing, by bioinformatic predictions or comparative genomics relying on the conservation of miRNAs among species.[27] There are estimates, in part based on computational methods, that there are as many as 1000 miRNA genes in the mammalian genome[28–30] which regulate 30% of the protein coding genes.[31] Hence, there will be few cellular processes that are not affected by miRNAs.

3. MicroRNA biogenesis and mode of action

MiRNA genes are found dispersed across the genome either as single genes or, for a considerable part, in gene clusters. The latter give rise to large polycistronic transcripts from which the individual miRNAs are processed. Some miRNA genes are located in intergenic regions but at least 50% are found in defined transcription units, i.e. introns or exons of both protein coding and long non-protein coding transcripts, and are consequently co-transcribed with the gene in which they reside.[32–34]

Fig. 1 gives a schematic overview of miRNA biogenesis and mode of action. MiRNA genes are transcribed by RNA polymerase II forming long – up to several kb – primary miRNAs (pri-miRNAs) that contain a 5′ CAP structure and are polyadenylated at their 3′ end. These pri-miRNAs contain one or more stem–loop or hairpin structures of ~70 nucleotides that are recognised and cleaved by the nuclear 650 kDa microprocessor complex consisting of a ds-RNA specific RNase III endonuclease Drosha and the ds-RNA binding protein, the DiGeorge syndrome critical region gene 8 (DGCR8), in humans or Pasha in flies and *C. elegans*.[35–37] The resulting precursor miRNA (pre-miRNA), a 60–100 nucleotide RNA hairpin intermediate with a two nucleotide 3′ overhang, is then shuttled to the cytoplasm by the nuclear export factor Exportin-5 and its cofactor Ran-GTP.[38,39] In the cytoplasm, the pre-miRNA is cleaved by a second RNase III endonuclease Dicer with its ds-RNA binding partner, the immunodeficiency virus (HIV) transactivating response RNA binding protein (TRBP), in humans or Loquacious (Loqs) in flies, yielding a ~20–25 nucleotide imperfect miRNA duplex.[40–42] Next TRBP recruits the human argonaute protein hAgo2 (EIF2C2) to the Dicer complex, thus forming a minimal RNA-induced silencing complex (RISC).[43–46] The essential core components of the RISC are members of the argonaute family of proteins which contain two conserved RNA-binding domains: the PAZ domain which binds the single-stranded 3′ end of the miRNA and the PIWI domain which structurally resembles ribonuclease-H and which interacts with the miRNA guide strand at its 5′ end.[47,48] Molecular characterisation of the RISC from various organisms suggests the presence of additional proteins like gemin 4 and the DEAD-box RNA helicase gemin 3,[49,50] the fragile X mental retardation protein (FMRP),[51] MOV10, another putative DEAD-box helicase and TNRC6B[52] and PACT, a ds-RNA binding protein.[53] The precise order of RISC assembly, the proteins and factors involved as well as the function of the additional proteins within the complex is still unclear. In mammals there are four argonaute proteins (Ago 1–4). Ago2, also known as slicer, is exceptional in the sense that it is the only argonaute protein with endonucleolytic activity, and capable of cleaving target sequences. As only one of the miRNA duplex strands is capable of functioning as guide strand directing the RISC to the 3′ UTR of target mRNAs, on the basis of sequence complementarity between the guide RNA and the mRNA, the passenger strand is removed.[54,55] The strand of the duplex with the weakest base-pairing at its 5′ terminus is selected as guide strand. Ago2 clearly plays a role in the process of siRNA passenger strand selection and degradation, but in the case of miRNAs that contain mismatches between guide and passenger strand a slower backup pathway dissociates and destroys the passenger strand.[54] Target mRNA cleavage, followed by mRNA degradation, only takes place if the miRNA has a near-perfect complementarity to its target sequence and commonly occurs in plants[56] and occasionally in animals.[57,58] When a mammalian miRNA pairs partially with its mRNA target, usually in its 3′ UTR, it cannot bring about mRNA cleavage but instead causes transcriptional silencing.[59–61] The binding of a single miRNA at a single site in the 3′ UTR is usually insufficient to measurably

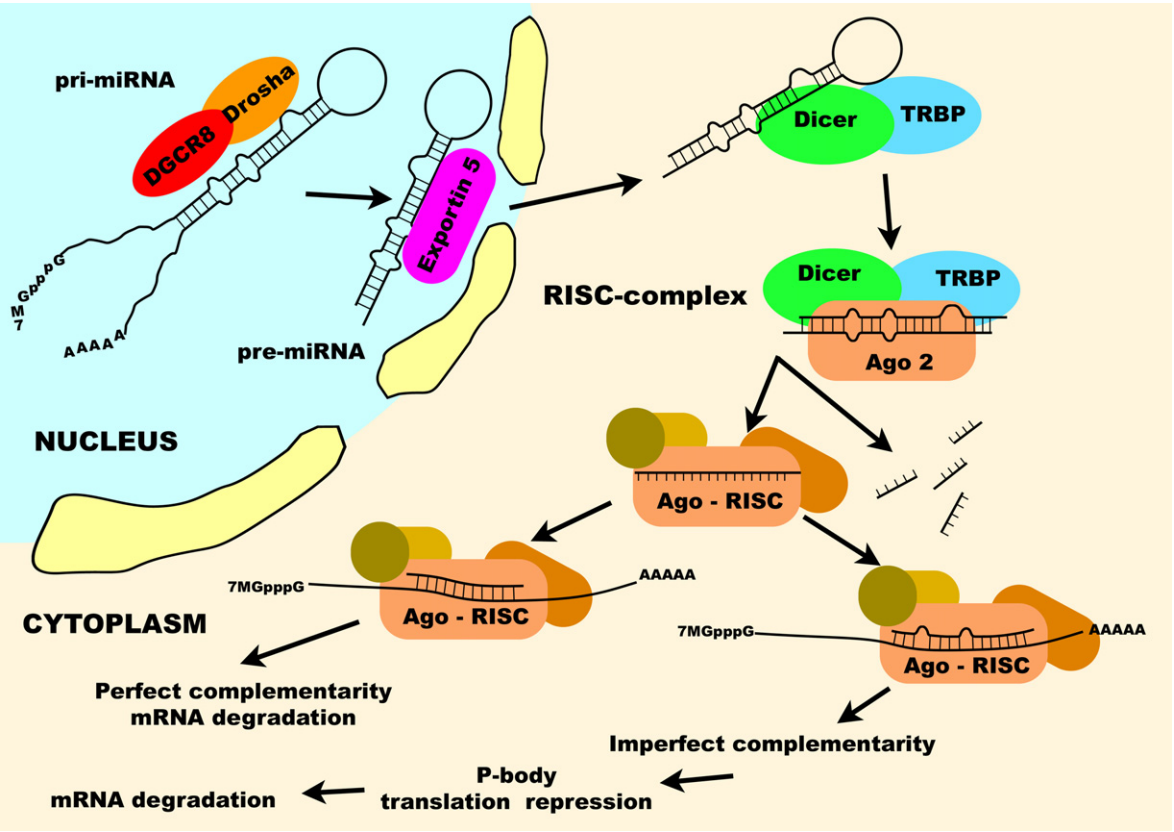

Fig. 1 – Schematic overview of the biogenesis and mode of action of mammalian microRNAs. MicroRNA genes are transcribed by RNA polymerase II either from their own promoter or from the promoter of the gene in which they reside. The capped and poly-adenylated transcript designated primary-miRNA (pri-miRNA) can be up to several kilobases in length and contain one or more miRNAs. The pri-miRNA folds into a characteristic stem–loop structure from which mature miRNAs are generated in two sequential processing steps. First the pri-miRNA is recognised and cleaved by a nuclear RNase III endonuclease called Drosha and its RNA binding partner DGCR8 (together called the microprocessor complex) resulting in the formation of a 60–70 nucleotide precursor miRNA (pre-miRNA) with a two nucleotide 3′ overhang. Exportin-5 and Ran-GTP are involved in the transport of the pre-miRNA to the cytoplasm where a second endonucleolytic cleavage occurs mediated by Dicer, in association with its RNA binding partner TRBP, generating a double-stranded RNA that is recruited to the RNA-induced silencing complex (RISC), a multiprotein complex containing at least one of the members of the argonaute family notably Ago2. Subsequently one of the strands, the passenger strand, is removed and degraded and the remaining strand, the mature miRNA, guides the RISC to target mRNAs that – depending on the level of complementarity between the miRNA and target sequence – are either translationally repressed or directly degraded. Most mammalian miRNAs bind in the 3′ UTR of their targets displaying mismatches due to imperfect complementarity. MiRNA binding leads to translational silencing. The exact molecular mechanisms by which translation can be inhibited are not yet completely clear. Recently, the so-called processing or P-bodies were singled out as sites where translational repression can occur. Target mRNAs were found to sequester in the cytoplasmic P-bodies, in a miRNA dependent fashion, away from the ribosomes thereby precluding their translation. In addition to a storage facility for mRNAs, P-bodies also contain the enzyme complement to degrade mRNAs possibly explaining why mammalian miRNAs cause a decrease in the mRNA levels of some targets. See text for further details.

block translation. Instead several miRNAs, either identical or different, must bind to the same target for translational repression to occur.[11] In this respect a surprising finding by several groups was that miRNAs – despite imperfect base-pairing with target sequences – cause not only translational repression but also degradation of a considerable number of target mRNAs.[25,62,63] Lim et al. reported that when the tissue-specific *miR*-1 (heart and skeletal muscle) or *miR*-124 (brain) precursors were transfected into HeLa cells, the mRNA expression profile shifted to that of muscle and brain, respectively. This implies that tissue-specific miRNAs affect many transcripts and help to generate and maintain tissue identity. Notably a couple of hundred mRNAs were downregulated already 12–24 h after transfection, probably a result of a direct association of the transfected miRNAs with binding sites in the 3′ UTR of the respective mRNAs.[25]

How do miRNAs affect translational efficiency?[64,65] Available evidence supports multiple mechanisms for RISC-miRNA mediated translational silencing inhibiting either the initiation or elongation of protein synthesis by the ribosomes. Alternatively, protein synthesis may proceed normally after which the protein is immediately proteolytically degraded.

Recent reports indicate that translationally silenced mRNAs are sequestered, away from the translational machinery, at distinct cytoplasmic sites, the so-called processing or P-bodies.[66–68] At these locations mRNAs accumulate that are destined for storage or decay. In order to perform the latter task P-bodies contain the decapping enzymes Dcp1/Dcp2, the 5′–3′ exonuclease Xrn1 and de-adenylation activity. Both hAgo1 and hAgo2 could be detected in P-bodies in a miRNA dependent manner.[67,68] In the absence of small RNAs or when *Ago* mutants were used unable to bind miRNAs, the argonaute proteins remained dispersed in the cytoplasm. The GW182 protein, a subunit of the P-body, interacts with the Ago proteins; knockdown of *GW182* interferes with the integrity of the P-body and impairs translational silencing.[69] Relocation of the mRNA to P-bodies inhibits translation and consequently, because of the mRNA degradation enzymes present in the P-bodies, may also affect mRNA levels. Translational repression is not always linked to mRNA degradation.[59,70,71] In fact a translational repressed mRNA in the P-body can be released again in response to the right stimuli and re-enter the translational machinery indicating that the process of miRNA directed translational repression can be reversible.[70]

4. The identification of microRNA targets

The fact that in animals most miRNAs have only a limited complementarity to their target sequences puts a challenge on the identification of potential target mRNAs using computational analyses. Only 6–7 nucleotides, usually nucleotides 2–8, the so-called 'seed sequence' of the 21 nucleotides or so comprising a miRNA, have been shown to be critical and in some cases sufficient for target silencing.[31] Various computer algorithms have been developed and aimed at identifying putative target genes for miRNAs.[72–76] Several target prediction programs for vertebrate miRNAs are available online like miRanda (http://www.microrna.org/miranda.html),[73] TargetScan/TargetScanS (http://genes.mit.edu/targetscan or http://www.targetscan.org),[31] PicTar (http://pictar.bio.nyu.edu)[74] and the mirBase (http://microrna.sanger.ac.uk/targets/v2). From these analyses it follows that most miRNAs have multiple targets ranging from dozens to hundreds and that target mRNAs may bind multiple miRNAs. Clearly, additional experimental verification is required before an mRNA can be considered a genuine target for a certain miRNA. Currently only few miRNAs are functionally annotated (see Table 1).

5. Function of miRNAs

The crucial importance of miRNAs for the development of vertebrates is best exemplified by the fact that depletion of components of the miRNA processing pathway, interfering with the maturation of miRNAs, is not compatible with life. Deletion of *Dicer* by knockout approaches causes embryogenesis to stall in an early developmental phase resulting in lethality.[77,78] *Dicer1*$^{-/-}$ mice display a developmental arrest at day 7.5 with embryos being small, appearing morphologically abnormal and devoid of pluripotent stem cells.[77] *Dicer1*$^{-/-}$ zebrafish embryos seem normal during the first week, probably due to residual activity of maternal Dicer1, but exhibit a developmental block at day 8 after fertilisation and most embryos die after two weeks.[78] Likewise knockdown of *TRBP*, Dicer's interaction partner, leads to destabilisation of Dicer and loss of miRNA biogenesis.[43] As expected Dicer1 deficient murine embryonic stem (ES) cells are defective in differentiation and the embryoid bodies fail to differentiate into the three germ layers and stop growing after 8 days of culture.[79] Recently *DGCR8* knockout murine embryonic stem cells were generated in which miRNA biogenesis was severely impaired.[80] Similar to *Dicer1* knockouts *DGCR8*$^{-/-}$ embryos also arrest early in development. Interestingly, and clearly different from *Dicer1* knockout ES cells, on induction of differentiation the *DGCR8*$^{-/-}$ ES cells retain their pluripotency and continue to grow and differentiate even after 16 days, suggesting that Dicer-1 has additional miRNA-independent functions in ES cell differentiation.[80]

In recent years, miRNAs have been shown to be involved in a variety of cellular processes mainly dealing with developmental and metabolic processes: cell proliferation, cell differentiation, developmental timing, flower development (plants), fat metabolism, apoptosis, insulin secretion, stem cell maintenance, neuronal patterning, and haematopoietic lineage differentiation see for reviews, e.g.[11,81–84]. Table 1 lists the mammalian miRNAs that are associated with defined biological processes and some of the target genes through which they exert their regulatory function.

As miRNAs are an integral part of the regulatory networks at work in cells, a perturbed miRNA function or altered miRNA expression may – either in a severe or subtle manner – disorganise cellular processes and eventually cause or contribute to disease (see review[85]). Recently, it was reported that mutations in a gene called *Slit and Trk-like 1* (*SLITRK1*) were associated with Tourette's syndrome (TS), a developmental neuropsychiatric disorder characterised by obsessive-compulsive symptoms.[86] A one base deletion was found in the coding region of *SLITRK1* which gave rise to a frame shift and truncated protein. Interestingly another sequence variation, which was found twice in a group of 174 unrelated TS patients and was not present in 3600 control chromosomes, mapped to the 3′ UTR of *SLITRK1* right in the predicted binding site for *miR-189*. The mutations probably arose independently as they occur in two different haplotypes. In brain regions implicated in TS both *miR-189* and *SLITRK1* are co-expressed and it was experimentally verified that *SLITRK1* is regulated by *miR-189*. The variant target site in which a G:U wobble base-pair is replaced by an A:U Watson–Crick pairing at position 9 in the miRNA binding domain was demonstrated to have a stronger regulatory effect. It is hypothesised that an altered interaction of the *SLITRK1* mRNA with *miR-189* contributes to TS in patients carrying the 3′ UTR mutation. A detailed analysis of the human single nucleotide polymorphisms (SNP) locations on the genome revealed that gain or loss of potential miRNA target sites in the 3′ UTR of genes occurs frequently.[82] Kloosterman and Plasterk report that there are 2490 SNPs that create a new potential miRNA target site and 2597 SNPs that destroy a target site which may as a consequence drastically modify gene regulation. More subtle variations in miRNA–mRNA target interaction dynamics can be the basis of phenotypic differences and possibly susceptibility for disease.

Table 1 – Mammalian microRNAs associated with defined biological functions

MicroRNA	Biological function	mRNA targets	References
miR-1	• Cardiomyocyte/skeletal muscle differentiation and proliferation	• *HAND2* • *HDAC4*	[157–159]
miR-7b	• AP-1 regulated processes	• *FOS*	[160]
miR-10a	• Megakaryocytopoiesis	• *HOXA1*	[161]
miR-15a miR-16-1	• Regulation of granulopoiesis • Regulation B lymphocyte survival • Cell cycle progression	• *BCL2* • Multiple targets involved in cell cycle (miRNA-16 family)	[71,114,116,162]
miR-16	• AU-rich element (AREs)-mediated mRNA stability	• Several ARE containing mRNAs	[163]
miR-17-5p[a] miR-19b miR-20 miR-93 miR-141 miR-199a-b miR-200a-c miR-429	• Skin morphogenesis		[164,165]
miR-27b	• Regulation drug metabolising enzymes	• *CYP1B1*	[166]
miR-32	• Antiviral defence	• Retrovirus PFV-1	[167]
miR-103	• Cell cycle progression	• Multiple targets involved cell cycle	[162]
miR-122	• Regulator of cholesterol and fatty acid metabolism • Facilitates replication of hepatitis C virus	• Hepatatis C virus • *SLC7A1* (CAT-1)	[70,152,153,168,169]
miR-130a	• Megakaryocytopoiesis	• *MAFB*	[161]
miR-132	• Neuronal morphogenesis	• *P250GAP*	[170]
miR-132 miR-146a-b miR-155	• Regulation innate immune response • Regulation macrophage inflammatory response	• *TRAF6* • *IRAK1*	[171,172]
miR-133	• Myoblast/skeletal muscle differentiation and proliferation	• *nPTB* • *SRF*	[157,173]
miR-134	• Regulation of dendritic spine development	• *LIMK1*	[174]
miR-142-5p	• Haematopoietic differentiation (B lymphoid and myeloid lineages)		[175]
miR-143	• Adipocyte differentiation	• *ERK5*	[176]
miR-155	• Angiotensin II related processes	• AT_1R	[177]
miR-181	• Haematopoietic differentiation (B-cell differentiation) • Myoblast differentiation	• *HOXA11*	[175,178]
miR-196a	• Developmental patterning	• *HOXB8*	[58,179,180]
miR-221 miR-222	• Erythropoiesis • Angiogenic activity SCF	• c-*KIT*	[181,182]
miR-223	• Haematopoietic differentiation (myeloid lineage) • Regulation granulopoiesis	• *NFI-A*	[71,161,175]
miR-375	• Regulation of insulin secretion	• *MTPN* (co-regulated by let-7 and miR-124)	[74,183]

Abbreviations: ARE, AU-rich element; AT_1 R, angiotensin II type 1 receptor; BCL2, B-cell lymphoma 2; c-KIT, stem cell factor receptor; CYP1B1, cytochrome P450 1B1; ERK5, extracellular signal regulated kinase 5; FOS, v-fos FBJ murine osteosarcoma viral oncogene homologue; HAND2, hand transcription factor; HDAC4, histone deacetylase 4; HOXA1, HOXB8, HOXA11, homeobox A1, B8 and A11; IRAK1, IL-1 receptor associated kinase; LIMK1, Lim domain containing protein kinase 1; MAFB, v-maf musculoaponeurotic fibrosarcoma oncogene homologue B; MTPN, myotrophin; NFI-A, nuclear factor I/A; nPTB, neuronal polypyrimidine tract-binding protein; PFV1, primate foamy virus type 1; p250GAP (RICS), Rho GTPase-activating protein; SCF, stem cell factor; SLC7A1, high affinity cationic amino acid transporter (CAT-1); SRF, serum response factor; TRAF6, TNF receptor-associated factor 6.

a A more extensive list of miRNAs differentially expressed in skin (epidermis and hair follicle) is given by Yi et al.[165] Note that *miRs-17-5p, -19b, -20, -93, -141, -200a-c, -429* are highly expressed in the epidermis, whereas *miR-199a-b* is found exclusively in hair follicles.

There is also evidence, albeit circumstantial, that links the miRNA processing machinery to cancer. The human argonaute genes hAgo1, 3 and 4 are located at 1p34-35 a chromosomal region often lost in Wilms' tumours.[87] HIWI, the human orthologue of the testis-specific murine PIWI protein that binds a new class of germ-line specific small non-protein coding RNAs (26–31 nucleotides) called PIWI interacting RNAs (piRNAs),[89–93] is linked to the development of testicular germ cell tumours and is overexpressed in most testicular seminomas.[94] Furthermore, reduced expression of Dicer is associated with poor prognosis in non-small cell lung carcinomas[95], whereas Dicer upregulation was shown in prostate adenocarcinoma.[96] Also in breast cancer, ovarian cancer and melanoma copy number abnormalities of *Dicer1* and *Ago2* have been reported.[97] Often mentioned in this respect is the DiGeorge syndrome, characterised by neonatal hypocalcaemia arising from parathyroid hypoplasia, thymic hypoplasia and outflow tract defects of the heart, resulting from a monoallelic deletion of chromosomal region 22q1.2, the so-called DiGeorge syndrome chromosome region or DGCR. One of the approximately 30 genes in the 1.5 Mb deleted region and perhaps partly responsible for the complex phenotype seen in DiGeorge syndrome may be the DGCR8, the ds-RNA binding partner of Drosha. Recent evidence, however, focusses on Tbx1 which is a transcription factor that seems responsible for typical features of the DiGeorge syndrome.[98–101]

6. MicroRNAs and cancer

Enhanced proliferation and dysregulated cell death are hallmark traits of the cancer cell.[102] Several of the first miRNAs described in *C. elegans* (*lin-4*) and *Drosophila* (*bantam*, *miR-14*, *miR-2/6/11/13/308*) were shown to affect just these cancer relevant pathways.[14,103–105] A large study involving the knockdown of 90 different miRNAs by anti-sense oligonucleotides also indicated multiple miRNAs that affect cell proliferation and apoptosis in HeLa and A549 cells.[106] Furthermore, miRNA profiling experiments revealed that many miRNAs are abnormally expressed in clinical cancer samples (see Table 2 and references therein). From the analysis of the expression profile of 228 miRNAs in six different solid cancers, a subset of 21 commonly deregulated miRNAs was selected, including miR-21, the miR-17-92 cluster and miR-191, that were overexpressed in the majority of cancer types examined.[107] It is proposed that the changed expression of these 21 miRNAs reflects commonly disturbed regulatory pathways in cancer cells. Finally, more than 50% of the miRNA genes are frequently found at cancer associated regions and fragile sites.[108] Zhang et al. using high resolution array based comparative genomic hybridisation (CGH) showed that a total of 41 miRNA genes, approx. 15% of all genes examined, exhibit gene copy number changes in three major cancer types, i.e. breast, ovarian cancer and melanoma, with 26 showing a copy number gain and 15 a copy number loss.[97]

Alterations in miRNA expression may promote tumour formation by modulating the functional expression of critical genes involved in tumour cell proliferation or survival. This does not mean that all miRNAs whose expression is perturbed are directly – in a causal way – involved in cancer progression and/or tumourigenesis. Many changes occur in cancer cells that, in a direct or indirect manner, may influence miRNA expression, e.g. genomic rearrangements, abnormalities in miRNA processing genes or proteins, and the disrupted epigenetic regulation of miRNAs.[109] Nevertheless, a number of miRNAs have been identified that appear to function as oncogene or tumour suppressor. These so-called oncomirs[110] are usually found to be aberrantly expressed in various malignancies of different histogenetic origin and are often located in genomic regions that are deleted, amplified or mutated in cancer cells. In addition, in *in vitro* and *in vivo* models the tumour suppressing or promoting activity was demonstrated and at least some relevant mRNA targets have been pointed out (see for recent reviews, e.g.[109–113]).

7. MicroRNAs with tumour suppressing activity

7.1. *MiR-15a and miR-16-1*

Initial evidence that miRNAs can play a role in cancer came from Croce and co-workers who were studying the molecular pathogenesis of chronic lymphocytic leukaemia (CLL), which is the most frequently encountered form of adult leukaemia in the Western World. In the majority of B-cell CLL (B-CLL) cases a genomic loss, either hemizygous or homozygous, of 13q14, is observed. Deletion analysis disclosed that the clustered *miR-15a* and -16-1 genes are the only genes within a small (30 kb) common region on 13q14 which is lost in CLL patients.[114] Accordingly, both miRNAs were found to be deleted or downregulated in more than 65% of B-CLL patients, suggesting that these miRNAs play a causative role in the pathogenesis of the disease.[114] More extensive miRNA profiling studies in B-CLL and normal $CD5^+$ B-cells confirmed the low expression or absence of *miR-15a* and -16-1 expression and identified two miRNA signatures corresponding to two distinct clusters of CLL associated with the presence of Zap70, a predictor of early disease progression.[115] Similarly, in 50% of mantle cell lymphomas, 16–40% of multiple myeloma and 60% of the prostate cancer deletions of 13q14 occur.[114] Interestingly, Cimmino et al. described that the anti-apoptotic Bcl2 is one of the targets of *miR-15a* and -16-1 as the expression of these miRNAs is inversely correlated with Bcl2 levels.[116] Both *miR-15a* and *miR-16-1* were shown to be capable of downregulating a reporter construct containing the *BCL2* 3′ UTR. Furthermore, *BCL2* translation repression by these miRNAs induced apoptosis in a leukaemia cell line. In conclusion, the loss-of-function of *miR-15a* and *miR-16-1* in CLL, and perhaps other cancers as well, may contribute to malignant transformation by upregulating Bcl2 thereby preventing apoptosis. Hence both miRNAs seem to act as tumour suppressors. It is very likely that *miR-15a* and *miR-16-1* modulate the expression of other genes as well some of which may also be relevant for cancer.

7.2. *Let-7 family*

Let-7 seems to fulfil a critical role in the pathogenesis of lung cancer as its expression was found to be reduced in non-small cell lung cancer (NSCLC)[117] and lower *let-7* levels corre-

Table 2 – Cancer-related human microRNAs[a]

Cancer type	MicroRNA	Up/down regulation	mRNA targets/biological function	References
Breast cancer	miR-10b	–	• *AIB1* (miR-17-5p)/growth hormone signalling pathways • ? (miR-21)/apoptosis	[107,127,128,184]
	miR-21	+		
	miR-29b-2	+		
	miR-17-5p	–		
	miR-125b-1,2	–		
	miR-145	–		
	miR-146	+		
	miR-155/BIC[b]	+		
Malignant lymphoma	miR-17-92 cluster[c]	+	• *E2F1* (miR-17-5p and miR-20a)/c-MYC cellular proliferation • *E2F2*, *E2F3*(miR-20a)/cellular proliferation, apoptosis	[122–126,136,137,139,185]
	miR-155/BIC	+		
B-CLL	miR-15a/16-1 cluster	–	• *BCL2*(miR-15a /16-1)/apoptosis	[114,116]
Burkitt lymphoma	miR-155/BIC	+/–[d]		[138,186]
Cholangiocarcinoma	miR-21	+	• *PTEN*(miR-21)/survival signalling • *CLOCK* (miR-141)? • *PTPN12* (miR-200b)?	[132]
	miR-141	+		
	miR-200b	+		
Colorectal cancer	let-7 familiy	–	• *RAS* (let-7-a1) • *TSP1* (miR-17-92 cluster) • *CTGF* (miR-17-92 cluster)	[107,120,187–190]
	miR-10a	+		
	miR-17-92 cluster	+		
	miR-20a	+		
	miR-24-1	+		
	miR-29b-2	+		
	miR-31	+		
	miR-96	+		
	miR-133b	–		
	miR-135b	+		
	miR-143	–		
	miR-145	–		
	miR-183	+		
Follicular thyroid carcinoma	miR-197	+	• *ACVR1*, *TSPAN3* (miR-197)? • *EFEMP2* (miR-346)?	[191]
	miR-346	+		
Glioblastoma	miR-21	+	• ? (miR-21)/apoptosis	[129,192]
	miR-128	–		
	miR-181a-c	–		
	miR-221	+		
Hepatocellular carcinoma	miR-18	+		[193]
	miR-125a	–		
	miR-195	–		
	miR-199a	–		
	miR-200a	–		
	miR-224	+		
Neuroblastoma	miR-34a	–	• *E2F3* (miR-34a)/apoptosis • ? (miR-184)/apoptosis	[194,195]
	miR-184	–[e]		
	multiple miRNAs	+/–[f]		
NSCLC	let-7 family	–	• *HRAS*, *KRAS*, *NRAS* (let-7 family)/oncogenic transformation	[107,117,118,120,196]
	miR-17-92 cluster	+		
	miR-21	+		
	miR-126*	–		
	miR-155/BIC	+		
	miR-200b	+		
	miR-205	+		
	miR-210	+		
Pancreatic cancer	miR-21	+		[107,130,131]
	miR-24-2	+		

(*continued on next page*)

Table 2 – *continued*

Cancer type	MicroRNA	Up/down regulation	mRNA targets/biological function	References
	miR-100	+		
	miR-103-1,2	+		
	miR-107	+		
	miR-125b-1	+		
	miR-155/BIC	+/–[g]		
Papillary thyroid carcinomas	miR-146b	+	• *KIT* (miR-221 and 222)/cell differentiation and growth	[144,181,197]
	miR-181b	+		
	miR-221	+		
	miR-222	+		
Pituitary adenomas	miR-15a/16-1 cluster	–		[198]
Prostate cancer	let-7d	+		[107]
	miR-128a	–		
	miR-195	+		
	miR-203	+		
Stomach cancer	miR-223	+		[107]
	miR-21	+		
	miR-218-2	–		
	miR-103-2	+		
Testicular germ cell tumours	miR-372	+	• *LATS2* (miR-372/373)	[121]
	miR-373	+		

Abbreviations: ACVR1, activin A receptor type1; AIB1, amplified in breast cancer 1; BCL2, B cell lymphoma 2; B-CLL, B cell chronic lymphocytic leukaemia; BIC, B-cell integration cluster; CLOCK, regulator circadian rhythms; CTGF, connective tissue growth factor; E2F1, E2F transcription factor 1; EFEMP2, EGF-containing fibulin-like extracellular matrix protein 2; KIT, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homologue; LATS2, large tumour suppressor homologue 2; NSCLC, non-small cell lung cancer; PTEN, phosphatase and tensin homologue deleted on chromosome 10; PTPN12, protein tyrosine phosphatase non-receptor type 12; RAS, RAS oncogene; TSP, thrombospondin-1; TSPAN3, tetraspanin 3.

a Listed are the most prominent differentially expressed microRNAs.

b *BIC* is a primary miRNA transcript containing *miR-155*.

c The *miR-17-92* cluster contains six miRNA genes, i.e. *miR-17*, *miR-18a*, *miR-19a*, *miR-20a*, *miR-19b-1* and *miR-92-1*.

d A relative high expression of *miR-155/BIC* in paediatric Burkitt lymphoma is reported by Metzler et al.[138] however, Kluiver et al.[186] demonstrated that compared to Hodgkin's lymphoma and normal lymphoid tissues paediatric/young Burkitt lymphoma patients have a low *miR-155/BIC* expression.

e Significantly downregulated in *MYCN* amplified tumours.

f A more extensive list of 32 microRNAs differentially expressed in neuroblastoma subtypes is given by Chen et al. [194]

g *MiR-155* was expressed in healthy pancreas but not, or to a lesser extent, in pancreatic endocrine tumours and pancreatic acinar cell carcinomas[131], whereas in pancreatic adenocarcinomas *miR-155* was found to be overexpressed.[130]

lated with shorter post-operative survival independent of the disease stage. Takimizawa et al. also observed that transient overexpression of *let-7* in the A549 lung adenoma cell line inhibited cell proliferation inferring that *let-7* is a tumour suppressor gene in lung tissue. Yanaihara et al. determined the miRNA expression profiles of 104 pairs of primary NSCLC and corresponding non-cancerous lung tissue.[118] Forty-three miRNAs were discovered to be differentially expressed between tumour and normal tissue and miRNA signatures were identified for the two most common NSCLC subtypes, i.e. adenocarcinomas and squamous cell carcinomas. A number of the miRNAs singled out, including *let-7* family members, are located in fragile sites and/or chromosomal regions that are often deleted in lung cancer. High *miR-155* or low *let-7a-2* expression was correlated to poor survival of adenocarcinomas stressing the prognostic value of miRNA profiling.[118] In contrast, Volinia et al. detected an upregulation of *let-7 g* and *let-7a-2* in NSCLC samples relative to healthy lung tissue after analysing 123 paired samples.[107] For reasons unclear, but probably of a technical nature, Volinia et al. did not observe the almost exclusive downregulation of miRNAs in cancer that was noted by Lu et al.,[119] but instead finds a large portion of the miRNAs overexpressed in cancer.

The *let-7* family of miRNAs in *C. elegans*, required for cell fate determination, consists of four developmentally regulated members, i.e. *let-7*, *miR-48*, *miR-84* and *miR-241*.[16,88] In search of the functions of the individual *let-7* family members, Johnson et al. performed a computational screen for genes with *let-7* complementary sites in their 3′ UTR.[120] It was found that *let-60*, the *C. elegans* orthologue of human *RAS*, is regulated by *let-7* and *miR-84*. Subsequently, they demonstrated that the human *RAS genes*, which are critical oncogenes, are regulated by human *let-7* family members. Ras proteins are membrane-associated GTPase signalling proteins that regulate cellular growth and differentiation. Mutations in the Ras family of proto-oncogenes, consisting of *KRAS*, *HRAS* and *NRAS*, are commonly found in 15–30% of the human tumours; inappropriate activation results in increased expres-

sion of Ras causing cellular transformation. The RAS genes have multiple, evolutionarily conserved, *let-7* complementary sites in their 3′ UTR subjecting the potential oncogenic proteins to *let-7* mediated regulation *in vitro*. Overexpression or depletion of *let-7* in human cancer cell lines results in decreased or increased Ras protein levels, respectively. Reporter constructs containing the *RAS* 3′ UTR are downregulated at the translational level by *let-7*. NSCLC tissue compared to normal adjacent tissue displays significantly reduced levels of *let-7* and by reciprocal correlation it displays increased levels of RAS, suggesting that disturbed *let-7* regulation of *RAS* is a step in NSCLC oncogenesis.[120] It will be interesting to see if other tumours with elevated and activated Ras levels like colorectal cancer, pancreatic cancer and bladder cancer also have reduced *let-7* levels and whether overexpression of *let-7* family members lowers Ras levels and reduces Ras mediated signalling. In humans, the *let-7* family consists of 11 members whose genes are localised at eight chromosomal locations; additional work is required to find out whether these related miRNAs have separate functions and/or show overlapping activities. Taken together, *let-7* seems to operate as a tumour suppressor in lung cancer by regulating the oncogene *RAS*.

8. MicroRNAs with oncogenic potential

8.1. MiR-372 and miR-373

Recently, *miR-372* and *miR-373* were implicated as oncogenes in human testicular germ cell tumours.[121] Using a functional genetic screen to find miRNAs that act as oncogenes in tumourigenesis, *miR-372* and *miR-373* were identified because of their ability to overcome the cellular senescence induced and maintained by oncogenic Ras and a functional wild-type p53 in primary human fibroblasts. It is known from the literature that loss of p53 allows the cells to overcome the growth arrest that characterises the senescence; however, *miR372* and *miR-373* do not target the *p53* mRNA. In fact cells expressing these miRNAs show normal p53 and p21 induction in response to Ras activation. Instead the two miRNAs somehow render the cells insensitive to the effects of a normal functioning p53 allele. Mechanistic studies revealed that *miR-372* and *miR-373* neutralise the p53-mediated cyclin-dependent kinase 2 (CDK2) inhibition and subsequent cell cycle arrest possibly through direct inhibition of the large tumour suppressor homologue 2 (*LATS2*) permitting proliferation and tumourigenesis. It is concluded that both miRNAs are potential oncogenes that participate in the development of human testicular germ cell tumours, a tumour type that rarely displays p53 loss-of-function, and act by numbing the p53 pathway, thus allowing tumourigenic growth in the presence of wild-type p53.[121]

8.2. MiR-17-92

The genomic locus 13q21-qter is often found amplified in haematological malignancies like diffuse large B-cell lymphoma, mantle cell lymphoma, follicular lymphoma and primary cutaneous B-cell lymphoma, but also in solid tumours such as glioma, NSCLC, bladder cancer, head and neck tumours and a number of other tumour types.[122] A common subregion of amplification at 13q31–32 is reported in diffuse large B-cell lymphomas and lymphoma cell lines. Several transcripts are derived from this genomic region including a polycistronic transcript from the *C13orf25* (Chromosome 13 open reading frame 25) gene that encodes the *miR-17-92* cluster encompassing six miRNAs: *miR-17*, *miR-18a*, *miR-19a*, *miR-20a*, *miR-19b-1* and *miR-92-1*. The amplification of 13q31–32 creates an overabundance of these miRNAs. The involvement of the *miR-17-92* cluster in tumour development was demonstrated when a truncated cluster *miR-17-19b*, cloned in a retroviral expression vector was expressed in haematopoietic stem cells (HSC). The HSC were derived from the foetal liver of *Eμ-myc* transgenic mice and give rise to B-cell lymphomas within 4–6 months when injected into lethally irradiated recipient mice. Co-expression of the *miR-17-19b* cluster and c-Myc resulted in the accelerated formation of highly malignant, disseminated lymphomas within approximately 2 months and implies that the *miR-17-19b* cluster promotes oncogenesis.[123]

O'Donnell presented proof that the transcription factor, c-Myc, regulates the *miR-17-92* cluster by mapping at least one binding site for c-Myc, approximately 1500 bp upstream of miR-17 located in intron 1 of *C13orf25*.[124] In addition two members of the *miR-17-92*cluster, i.e. *miR-17-5p* and *miR-20a*, were shown to negatively regulate the translation of the c-Myc target gene E2F1 encoding a transcription factor belonging to the familiy of E2F transcription factors (E2F1, E2F2 and E2F3) that play an essential role in the regulation of cellular proliferation and cell cycle progression.[124] It seems that the *miR-17-92* cluster, by decreasing the transcription factor levels of E2F1, tightly regulates c-Myc mediated cellular proliferation and in this sense can be considered to display a tumour suppressing activity. Interestingly, transcription of the *miR-17-92* cluster is not only controlled by c-Myc[124], but is also under influence of the transcription factors E2F1–3[125,126] whose translation is negatively regulated by *miR-20a*,[124,125] suggesting the existence of complex genetic connections controlling cellular proliferation.

8.3. MiR-21

MiR-21 is found to be highly expressed in numerous cancers like breast cancer,[127,128] glioblastoma[129] and pancreatic cancer.[130,131] Downregulation of *miR-21* by transfection of breast cancer or glioblastoma cell lines with anti-sense *miR-21* results in a reduced cell proliferation rate accompanied with an increased apoptosis.[128,129] Apparently, *miR-21* functions as an oncogene by inhibiting pro-apoptotic genes. Recently Meng et al. showed that *miR-21* is also overexpressed in malignant cholangiocarcinomas, a highly chemoresistant cancer type, knockdown of *miR-21* sensitised cholangiocarcinoma cell lines for treatment with gemcitabin, whereas transfection of non-malignant cholangiocytes with precursor *miR-21* made cells more resistant to gemcitabin.[132] Further studies indicate that *miR-21* targets the tumour suppressor *PTEN*, a phosphatase that antagonises the survival/growth promoting activity of the PI-3 kinase-Akt signalling pathway. The available data suggest that overexpression of *miR-21* leads to downregulation of Pten and a more active survival signalling through the PI-3 kinase-Akt pathway rendering the cells less susceptible for

apoptosis and cell cycle arrest and explaining the chemoinsensitivity of cholangiocarcinoma cells.[132] It remains to be seen to what extent Pten regulation by *miR-21* determines chemosensitivity and whether this also occurs in other cancer types that overexpress *miR-21*.

8.4. MiR-155

The *BIC* gene was first identified in an avian model as a common retroviral integration site in B-cell lymphomas induced by the avian leukosis virus and has been shown to cooperate with c-Myc in lymphomagenesis and erythroleukemogenesis.[133,134] Comparison of the *BIC* genes from human, mouse and chicken highlighted a stretch of 138 nucleotides with a 78% degree of identity corresponding to the *miR-155* precursor.[18,135] Both *BIC* and *miR-155* were found to be highly expressed in Hodgkin's, primary mediastinal and diffuse large B-cell lymphomas,[136,137] Burkitt lymphoma[138] and NSCLC[118]; a reduced expression was detected in pancreatic cancer.[107,131] A transgenic mouse model was generated that overexpresses *miR-155* under control of the V_H promoter–Ig heavy chain *E*μ enhancer (Eμ-miR-155) in B-cells.[139] The mice first establish a preleukaemic pre-B cell proliferation that predominantly manifests itself in spleen and bone marrow, and which subsequently evolves into a B-cell malignancy. The observed polyclonal expansion, apparently initiated by the expression of *miR-155*, can be considered an early event in oncogenesis, after which secondary genetic changes are necessary for full transformation. This is the first example of a transgenic mouse model in which targeted overexpression of a miRNA predisposes to cancer.

The question arises whether, in addition to being abnormally expressed, miRNAs themselves may harbour mutations that either impair their function or alter target specificity. Particularly, mutations in the seed sequence could have a severe impact by changing important regulatory control mechanisms. As mutations in mature miRNA sequences are likely to affect the regulation of multiple genes, one expects a strong negative selection against these mutations. Indeed the virtual lack of sequence variants in mature miRNAs underscores this argument. A germ-line mutation was observed in the *miR-15a* and -16-1 primary miRNA transcripts located 7 base-pairs after the 3′ end of *miR-16-1* close to the base of the hairpin structure but in a genomic region conserved in primates. The mutation, which was present in two out of 75 CLL patients examined, and which was not found in 160 healthy controls, resulted in a diminished *miR-16-1* expression both *in vitro* and *in vivo* and was associated with deletion of the normal allele.[140] Analysis of 173 human pre-miRNA genome regions of 96 subjects revealed 10 polymorphisms in pre-miRNA hairpin regions, most of which were located outside the mature miRNA sequence and had no effect on miRNA processing with the exception of a C to A polymorphism in the mature *miR-30c-2* that alters the target selection.[141] In two recent large scale screening, efforts no mutations were found in the mature miRNA sequences examined.[142,143] Chen et al. sequenced all 13 X-chromosomal pre-miRNAs in 464 patients with non-syndromic X-linked mental retardation, whereas Diederichs and Haber examined 15 cancer related miRNA genes in 91 cancer derived cell lines. In both studies sequence variants were detected in precursor as well as primary miRNAs but none was found to interfere with miRNA processing.[142,143] Conversely mutations or polymorphisms in the 3′ UTR of mRNAs may lead to loss or gain of miRNA target sites. Note that the acquisition of novel miRNA target sites is only biologically relevant if both miRNA and mRNA are expressed in the same cell type under the same conditions. Two single nucleotide polymorphisms in the 3′ UTR of the stem cell factor receptor c-Kit were described that are localised in the crucial recognition regions for miR-221 and -222 and the binding site for miR-146a and -146b.[144] These miRNAs are strongly upregulated in papillary thyroid carcinoma compared to normal thyroid tissue and may be the reason for the loss of *KIT* transcripts and Kit protein observed in the tumours. In at least 50% of the tumours examined, the downregulation of Kit seems to correlate with the presence of the SNPs, suggesting that miRNAs and Kit are involved in papillary thyroid pathogenesis.[144]

9. MicroRNAs and their use in the clinic

An important and compelling question is how these novel findings concerning miRNAs and their role in cancer gene regulation can be put to use in the clinic.[109,145–147] Firstly, miRNA expression profiles can be used to classify tumours according to their differentiation state and developmental origin[107,119] and may serve a diagnostic purpose.[148] In fact as shown by Lu et al., the miRNA expression profile based on the expression of only 200 miRNA genes successfully classified poorly differentiated tumours confirming in the majority of cases the clinical diagnosis whereas mRNA profiling, based on the expression of appr. 16,000 protein coding genes, failed to do so.[119] These findings suggest that miRNA profiling is far more informative than regular mRNA profiling. Secondly, prognostic miRNA expression signatures may be identified within tumour groups that predict, e.g. high progression risk, poor survival or presence of metastases.[118,131,140] Thirdly, miRNAs may be target or means for cancer prevention or therapeutic intervention. Consider, e.g. *let-7* or *miR-15a* and -16-1 whose loss of expression seems an essential step in the pathogenesis of lung cancer and B-CLL, respectively. If one would be able to induce *let-7* expression or re-express *miR-15a* and -16-1, one may be able to prevent the formation or progression of these types of cancers. Overexpression of *let-7* does indeed counteract the effects of activating mutations of KRAS in lung cancer.[120] Expression of *miR-15a* and/or *miR-16-1*, that target the anti-apoptotic *BCL2* gene, in tumours exhibiting high Bcl2 levels may initiate apoptosis and resensitise the cells for chemotherapeutic treatment.[116]

Other miRNAs may be upregulated in cancer cells, e.g. due to genomic amplification as is the case for the *miR-17-92* cluster in lymphomas. In these instances it may be beneficial to downregulate the overexpressed miRNAs which can be done by an anti-sense approach. 2′-O-methyl RNA oligonucleotides[149,150] or locked nucleic acid (LNA) modified oligonucleotides[151] complementary to miRNAs can effectively block miRNA function when transfected into cell lines causing a loss-of-function phenotype. The chemical adaptations of the RNA backbone aim to increase stability and withstand degradation in cell culture medium and/or serum. Strikingly anti-sense miRNAs also appear to work *in vivo* as demon-

strated by Krützfeldt et al. who reported that endogenous *miR-16*, *miR-122*, *miR-192* and *miR-194* in adult mice could efficiently and specifically be silenced by the intravenous injection of chemically modified, cholesterol-conjugated, single-stranded RNA analogues complementary to the mature miRNAs.[152] These antagomirs, as they are aptly named, consist of 2′-*O*-methyl modified nucleotides and the terminal nucleotides are coupled with phosphorothioate linkages. The cholesterol moiety at the 3′ end improves stability and half-life in serum and enhances cellular uptake. Dose-response, persistence of response and tissue distribution were some of the pharmacological parameters examined using antagomirs against the liver specific *miR-122*. After a total dose of 240 mg/kg, administered in three separate injections of 80 mg/kg per day, no *miR-122* signal could be detected on Northern blots. The complete loss of *miR-122* lasted for at least 23 days, implying that the miRNA is both functionally blocked and degraded. *MiR-122* was effectively targeted in all tissues except the brain, seemingly antagomirs cannot translocate across the blood–brain barrier. The inhibition of *miR-122* led to the upregulation and downregulation of hundreds of genes in the liver as judged by mRNA profiling studies, indicating that many genes can be repressed or activated in a direct or indirect manner by *miR-122*. The mRNA expression data revealed that *miR-122* participates in the regulation of the cholesterol biosynthetic pathway as at least 11 genes of this anabolic route showed reduced mRNA levels after treatment with *miR-122* antagomirs. A corresponding 44% drop in plasma cholesterol levels was observed in the treated mice see also[153]. Note that a *miR-122* knockdown supposedly leads to an upregulation of its direct mRNA targets, however, the mechanism by which mRNAs are downregulated upon *miR-122* inhibition is less clear. A possibility is that *miR-122* targets a transcriptional repressor common to the genes comprising the cholesterol biosynthetic pathway. Whatever the exact underlying causes are, these findings illustrate the complications that may arise upon systemic treatment, after all miRNAs have multiple targets and therefore variations in their expression levels will affect many different genes possibly causing gross alterations in cellular and tissue physiology. Another point of concern is the possible off-target effects, i.e. the silencing of unintended target miRNAs by anti-sense miRNAs. This may especially be difficult to prevent in case of closely related miRNAs. Nevertheless, the studies of Krützfeldt et al. do clearly show the therapeutic potential of synthetic anti-sense miRNAs and emphasise the usefulness of antagomirs for *in vivo* functional biological studies.[152]

Two interesting additional therapeutic approaches using miRNAs have been reported. First, Tsuda et al. who simply designed a synthetic miRNA to target a gene that is not normally controlled by miRNA.[154] The glioma-associated antigen 1 (Gli-1) is a transcription factor that functions in the sonic hedgehog signalling cascade, constitutive activation of which has been associated with the growth of a number of human malignancies. A miRNA was generated that specifically targets the 3' UTR of *GLI-1* and significantly inhibits the proliferation of Gli-1 positive ovarian and pancreatic tumour cell lines.[154] Second, miRNAs can also be used to facilitate other ways of treatment like gene therapy whose success is often hampered by the development of transgene specific immunity. Brown et al. developed a miRNA-regulated gene expression system in which a transgene is tagged with target sites for *miR-142-3p*, a miRNA that is specifically expressed in the haematopoietic lineage.[155] Transgene expression in haematopoietic cells, including the antigen-presenting cells (APCs) that contribute significantly to the induction of an immune response, is effectively silenced because of endogenous *miR-142-3p*. The lineage specific knockdown of the transgene, while allowing expression in non-haematopoietic cells, reduces the immunological side-effects of gene therapy as shown by the high-level stable expression of a transgene in immunocompetent mice.[155]

Ultimately, the successful use of the miRNAs machinery for cancer therapy relies on a profound insight in the biology of miRNAs as well as on technical issues such as the development of safe and efficient delivery systems that introduce miRNA expression vectors or specific anti-sense miRNAs into target tumour cells. In this respect, it is important to realise that the RNAi processing machinery, which is shared by both siRNA and miRNAs, can be overwhelmed due to the highly efficient delivery of a single siRNAs, or miRNA for that matter, leading to severe toxicities and even fatalities in animals.[156] Considering the rapid pace at which the npcRNA research is progressing, the next decade will undoubtedly bring us novel insights in the intricate relationship of miRNAs and tumourigenesis, and a better understanding of how these novel ribo-regulators fit into the complex regulatory networks operational in cancer cells.

Conflict of interest Statement

None declared.

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