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MicroRNAs and lncRNAs as Tumour Suppressors

Emanuela Boštjančič and Damjan Glavač

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1. Introduction

Cancer is one of the most serious diseases around the world and it is the third leading cause of death, exceeded only by heart and infectious diseases [1]. There are five major steps for cancer development: initiation, promotion, malignant conversion, progression, and metastasis [2]. Cancer is result of process, where somatic cells mutate and escape the controlled balance of gene expression and cellular networks that maintain cellular homeostasis, which normally prevent unwanted expansion. Perturbations in these pathways results in cellular transformation, where cancer cells differ from their normal counterparts in many characteristics, as is loss of differentiation, increased invasiveness, and decreased drug sensitivity [3-4]. There are six primary hallmarks of cancer: unlimited cell proliferation, autonomous growth without the need of external signals, resistance to growth inhibitory signals, escape from apoptosis the ability to recruit new vasculature and increased tissue invasion and metastasis [5]. The formation of cancer is therefore fundamentally genetic and epigenetic disease requiring accumulation of genomic alterations to inactivate tumour suppressor and activate proto-oncogenes [6]. These results in combined interaction of both tumour suppressors, that are not able to inhibit tumour development and protect cells against mutation that initiate transformation, and cancer inducers, which promotes cancer development as initiators of cellular transformation. When cells exhibit abnormal growth and loss of apoptosis, it usually results in cancer formation [2].

Genetic studies have revealed the mutational and epigenetic alterations of protein-coding genes that control DNA damage response, growth arrest, cell survival and apoptotic pathways [4]. Until recent years ago, the central dogma of molecular biology was that genetic information is stored in protein-coding genes with RNA as an intermediate between DNA sequence and its encoded protein [7]. Recent studies suggest that advanced stages of cancer are possessing more severe molecular perturbations and that this could be due to function of non-coding RNAs (ncRNAs), which were previously known only to have infrastructural functions (as

ribosomal RNA, transfer RNA, small nuclear and nucleolar RNA). Eukaryotic genomes are extensively transcribed into thousands of long and short ncRNAs, which are group of endogenous RNAs that also function as regulators of gene expression. They are involved in developmental, physiological as well as pathological processes [7,8].

However, in this review, the following characteristics of ncRNA in human cancers will be summarized: (i) the current understanding of the critical role that lncRNAs and miRNAs may play in cancer as tumour suppressors; (ii) outline current knowledge about some specific lncRNA and miRNAs and their target genes in cancer; (iii) highlight their potential as biomarkers for patho-histological subtype classification; and (iv) highlight their potential as biomarkers and as circulating biomarkers and therapeutic targets in cancer.

Since the majority of research regarding regulatory ncRNAs as tumour suppressor was performed on miRNAs and in lesser extend on lncRNAs/lincRNAs, will this review further focused on these two groups of ncRNAs.

2. Brief overview of non-coding RNAs (ncRNAs)

Classification

Of transcribed eukaryotic genomes, only 1-2 % encode for proteins, whereas the vast majority are ncRNAs that are in more or less functional transcripts. The regulatory ncRNAs are important regulators of gene expression in many eukaryotes and are involved in a wide range of functions in eukaryotic biology [8,9].

Based on their function, ncRNAs can be divided into two groups. First is infrastructural group, with ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), small nuclear RNAs (snRNAs) and small nucleolar RNAs (snoRNAs). Second is regulatory group, with microRNAs (miRNAs), piwi-interacting RNAs (piRNAs), small-interfering RNAs (siRNAs), long non-coding RNAs (lncRNAs), large intergenic non-coding RNAs (lincRNAs), promoter-associated small RNAs (PARs), repeat-associated short interfering RNAs (rasiRNAs) and enhancer RNAs (eRNAs) [8,9]. Recent findings suggest that some structural ncRNAs (e.g. snoRNAs) not only have infrastructural function but have regulatory as well [8].

Based on length, the regulatory ncRNAs can be divided in two groups: larger than 200 nucleotides (nt) are lncRNA, lincRNA, eRNA, whereas the others are smaller than 200 nt, with exception of PARs that are 16-30 nt long or up to 200 nt. Distinct classes of small RNAs are distinguished by their origins, and these are: snRNAs, snoRNAs, miRNAs, piRNAs, siRNAs, and rasiRNAs [8,9].

miRNAs and snoRNAs share similarities in processing pathways and protein interaction partners, genomic organization and location, as well as levels of conservation. However, similarities in sub-cellular localization have been also observed, since large proportion of human mature miRNAs have been detected in the nucleus as well as a subset of small RNAs derived from snoRNAs have been detected in the cytoplasm [10].

Functional role

The most widely studied and characterized of all the regulatory ncRNAs are miRNAs. The roles of regulatory ncRNAs, other than miRNAs, in the mediating transcriptional regulation, chromatin remodelling, post-transcriptional regulation, and other processes are less well understood. The contexts of gene regulation by ncRNAs in non-human systems provided insights into how these processes could function in human cells. Regulatory ncRNAs are involved in diverse cellular pathways, such as development and stem cell maintenance, response to stress and environmental stimuli, regulating chromatin structure and remodelling, chromosome architecture and genome integrity, transcription (positive or negative impact), and post-transcription processing (splicing, transport) and most commonly mRNA stability (translation, degradation) [8,9]. Some ncRNAs trigger different types of gene silencing that are collectively referred to as RNA silencing or RNA interference [11].

RNA interference (RNAi)

RNAi is RNA-guided regulation of gene expression, historically known by other names, including post-transcriptional gene silencing. It is believed to be an evolutionary conserved mechanism in response to presence of foreign dsRNA in the cell. A key step in this silencing pathway is the processing of dsRNAs into short RNA duplexes of characteristic size and structure. The enzyme Dicer, which initiates the RNAi pathway, cleaves dsRNA to short double-stranded fragments of 20–25 base pairs (bp), named siRNAs. siRNAs usually possess perfect complementarity to the mRNA of target gene, thus causing its degradation. When the dsRNA is exogenous, coming from infection by a virus with RNA genome or laboratory manipulations, the RNA is imported directly into the cytoplasm where it is cleaved by the Dicer. On the other hand, the initiating dsRNA could be result of endogenously expressed RNA-coding genes from the genome. Some of small regulatory RNAs are processed in a similar way or with components of RNAi pathway [11].

2.1. Brief introduction to miRNAs

Genomic organization

miRNAs are endogenously expressed small (~22 nt), single-stranded ncRNAs. It is predicted that they constitute ~1-5 % of human genes [1,12] and in an update from August 2012, miRBase v19 was released with a list of 2019 unique mature human miRNAs. miRNAs are encoded as a single gene or gene clusters, with some of miRNA clusters being co-regulated and co-transcribed. Intergenic miRNAs are transcribed as an independent transcription unit, as a monocistronic, bicistronic or polycistronic primary transcripts [13]. Up to 60 % of currently known miRNAs are proposed to be from intronic sequences of either protein coding or non-coding transcription units and suggestion has been made that some miRNAs are also encoded in antisense DNA, which is not transcribed to the mRNA. Intronic miRNAs are preferentially transcribed in the same orientation as the host gene and are together with their host transcripts co-regulated and co-transcribed from the same promoter. They are processed from introns, as are many snoRNAs. Within the genome, there might be more than one copy of particular miRNA [13,14].

Biogenesis

miRNAs expression is determined by intrinsic cellular factors and diverse environmental variables [1]. As for protein-coding genes it is known, that regulation of miRNA transcription and expression depends on transcription factors and epigenetic mechanisms (e.g. p53, Myc, and myogenin). In general, from genes encoding miRNAs is transcription guided by RNA-polymerase II (Pol II). Resulting primary transcript (several hundred bases to several kilobases), named *pri-miRNA*, forms distinctive hairpin-shaped stem-loop secondary structure and contains poly-A tail and a cap, similarly to protein-coding mRNA. *pri-miRNA* is processed in the nucleus by Drosha, an RNase III enzyme. The resulting 70-nt stem-loop structure called *pre-miRNA* with a 5' phosphate and 3' 2-nt overhang is imported into the cytoplasm by a transporter protein, Exportin 5. The double-stranded RNA portion of *pre-miRNA* is bound and cleaved by Dicer, another RNase III enzyme, which produces a miRNA:miRNA* duplex (a transient intermediate in miRNA biogenesis, 20–25 nt). One of the two strands of each fragment is together with proteins argonaute (Ago), incorporated into a complex called the miRNA-containing ribonucleoprotein complex (miRNP). It is believed that the *guide strand* is determined on the basis of the less energetically stable 5' end. The resulting complex base-pair with complementary 3'-UTR mRNA sequences. The other strand, miRNA* is presumably degraded, although there are increasing evidence that either or both strands may be functional [2,9]. The schematic overview of canonical miRNA biosynthesis pathway has been represented elsewhere [15].

Numerous alternative pathways differing from canonical miRNA biogenesis pathway have been described recently and subset of several diverse longer non-coding RNAs can serve as precursors for miRNAs [10,16]. As an example, intronic miRNAs presumably bypass Drosha cleavage, since through *pre-mRNA* splicing/debranching machinery is produced an approx. 60-nt hairpin precursor miRNA (*pre-miRNA*) that enter biogenesis pathway at the step of Exportin 5 [14]. However, some of the post-transcriptional mechanisms include miRNA editing, which is mechanism mediated by adenine deaminase of alteration of adenines to inosines, and not yet thoroughly studied regulations of miRNA, such as export step from nucleus or miRNAs turnover rate [17].

miRNAs mechanism

The functional role of miRNA varies, but the primary mechanism of miRNA action in mammals is believed to be base-pairing to 3'-UTR of target mRNA followed by inhibition of mRNA translation (when base pairing between these two molecules is incomplete) or deadenylation and degradation (perfect complementarity of miRNA:mRNA binding) [9]. Especially in animals, the primary mechanism of miRNA action is reducing mRNA translation and each miRNA can inhibit the translation of as many as 200 target genes. In addition, mRNA can be regulated by more than one miRNA. The cooperative action of multiple identical (multiplicity) or different miRNPs (cooperativity) appears to provide the most efficient translational inhibition. Additional mechanism to increase the specificity of miRNAs is combinatorial control of gene expression, which may be also provided by a set of co-ordinately expressed

miRNAs. Proteins or mRNA secondary structures could restrict miRNP accessibility to the UTRs, or may facilitate recognition of the authentic mRNA targets [12,13,18,19].

There is the prospect that some miRNA might specify more than just post-transcriptional repression [9,13]. miRNAs may also target promoter to regulate transcription through epigenetic mechanism. miRNAs have been paradoxically also shown to up-regulate gene expression by enhancing translation under specific conditions [9].

Biological function

Translational repression, as major mechanism of miRNAs, may in normal cell conditions occur in different ways: as switch off the targets, that is for mRNAs that should not be expressed in a particular cell type, the protein production is reduced to inconsequential levels; as fine-tuners of target expression, that is when miRNAs can adjust protein output for customized expression in different cell types; as neutralizers of target expression, that is when miRNAs act as bystanders, where down-regulation by miRNAs is tolerated or reversed by feedback processes [13]. Role of miRNA can be further divided in three paradigms: combinatorial control (defined as cooperativity), cell-to-cell variation, specific (tissue-specific and/or cell-type specific) and housekeeping functions [20].

Despite the large number of identified miRNAs, the scope of their roles in regulating cellular gene expression is not fully understood [11]. It is believed that miRNAs through negative gene regulation influence at least 50 % of genes within the human genome [9]. Expression profiling of many miRNAs in various normal and diseased tissues have demonstrated unique spatial and temporal expression patterns. Many miRNAs are important at distinct stages of development and have been found to regulate a variety of physiological and pathological processes [11]. miRNAs are involved in a numerous biological processes, such as stem cell division and developmental timing, proper organ formation, embryonic patterning and body growth, proliferation and differentiation, apoptosis, epithelial-mesenchymal-transition (EMT), cholesterol metabolism and regulation of insulin secretion, resistance to viral infection and oxidative stress, immune response etc. [2,11]. All these effects may occur by regulating or being regulated by the expression of signalling molecules, such as cytokines, growth factors, transcription factors, pro-apoptotic and anti-apoptotic genes [21]. With all different genes and expression patterns, it is reasonable to propose that every cell type at each developmental stage might have a distinct miRNA expression profile.

Defining miRNA targets and databases

Up to date, over 2000 human miRNAs have been identified and this number is still growing. All annotated miRNAs are collected in miRBase [22]. The first step in miRNA target identification is usually defining reciprocally regulated miRNA-mRNA or miRNA-protein. Since miRNAs target mRNA mainly by incomplete base-pairing, many computational methods have been recently developed for further identifying potential miRNA targets [23]. Most of these methods search for three criteria in predicting miRNA target genes: first, multiple conserved regions of miRNA complementarities within 3'-UTR of target mRNA (evolutionary conservation); second, interaction between seven consecutive nucleotides in the target mRNAs 3'-UTR and the 1-8 nt ("seed sequence") at the 5' miR-

NA end; third, stability of base pairing and predicted binding energy. Further complicating target site prediction in mammals is the fact that not all 3'-UTR sites with perfect complementarities to the miRNA seed nucleotides are functional. Moreover, mRNAs sites with imperfect seed complementarities can themselves be very good miRNA targets [24,25]. Bioinformatics is therefore much noisier and more prone to false positive and false negative predictions. Among many available programs for predicting mRNA targets for specific miRNA, none of these programs can be used as an independently approach for validating the targets, and all predicted targets must be validated *in vitro* and/or *in vivo*. Thus the gold standard for miRNA target identification is the experimental demonstration that a luciferase reporter fused to the 3'-UTR of the predicted target is repressed by over-expression of the miRNA and that this repression is abrogated by point mutation in the target sequences in 3'-UTR [26,27]. Finally, expression profiling in human disease gives the starting point for target verification/validation and association to disease prognosis and pathogenesis. All identified disease related miRNAs are listed in The human microRNA disease database (HMDD), where you can search for specific miRNA, for tissue expression of annotated miRNAs, and for disease related miRNAs [28].

2.2. Brief introduction to lncRNA

Genomic organization

lncRNAs are those longer than 200 nt, and many of them can also act as primary transcripts for the production of short RNAs [9]. It is estimated that total number of lncRNA transcripts, including new unexplored, is approx. 15000. Thousands of protein-coding genes in humans harbour natural antisense transcripts (approx. 61 % of transcribed regions show antisense transcription) belonging to the lncRNA, and majority of known lncRNAs in some way overlap protein-coding loci. All these data are giving the importance to lncRNA annotation [29].

Classification

lncRNAs can be classified according to their proximity to protein coding genes. There are five categories of lncRNAs: sense, antisense, bidirectional, intronic, intergenic. Just to mention a few of them, lincRNAs, a class of ncRNAs, exhibit a high conservation between different species; they both up- and down- regulate hundreds of gene expression and participate in the establishment of cell type-specific epigenetic states [9]. Further, ncRNAs were found expressed at enhancer regions, suggesting that some enhancer RNA is also transcribed with an average size of 800 nt; these transcripts are termed eRNAs. Studies propose a possible role for eRNAs as transcriptional activators, however, question remains whether such eRNAs are in fact a subset of the activating lncRNAs. Similar to eRNA, a novel diverse class of ncRNAs has been linked to the promoters, called PARs, ranging from 16-36 nt to 200 nt. It is suggested that they participate in the transcriptional regulation [9]. Most lncRNAs are characterized by low expression levels, low level of sequence conservation, by composition of poly-A tail and without poly-A tail as well as by spliced and un-spliced forms. They are believed to have nuclear localization, but can also accumulate in cytoplasm of cells [3].

Function

lncRNAs may act through diverse molecular mechanisms, and play regulatory as well as structural roles in different biological processes [3]. Many of the identified lncRNAs show spatial- and temporal-specific patterns of expression. Almost every step in the life cycle of genes – transcription, mRNAs splicing, RNA decay, and translation – can be influenced by lncRNAs. Generally lncRNAs have been implicated in gene-regulatory roles, such as chromatin dosage-compensation, imprinting, epigenetic regulation, cell cycle control, nuclear and cytoplasmic trafficking, cell differentiation etc. [7]. A number of studies suggest that lncRNAs are key components of the epigenetic regulatory network [4]. Two general modes of lncRNAs regulation seem to be important: interaction with chromatin remodelling complexes that promote silencing of specific genes; and modulation of splicing factors. Chromatin remodelling guided by ncRNAs contributes to the establishment of chromatin structure and to the maintenance of epigenetic memory. Various ncRNAs have been identified as regulators of chromatin structure and gene expression [30]. Additional mechanisms of action are yet to be revealed [3].

Database

The lncRNA database provides sequence, structural, and conservation evidence for multi-species lncRNAs, together with a list of lncRNAs that are experimentally known to interact with coding mRNAs, harbouring other short ncRNAs and other characteristics of specific lncRNA [29].

3. Involvement of ncRNA in cancer

Three major mechanisms are known to give rise to deregulated ncRNAs function, genetic alterations, epigenetic alterations, and in case of miRNAs, an aberrant miRNA biogenesis machinery. Since brief overview of first two mechanisms is described below, will be here mentioned only aberrant machinery of miRNA processing. Proteins involved in miRNA biogenesis (Drosha, Dicer, Ago) are deregulated in several cancers. Co-factors involved in miRNA biogenesis can be mutated causing consequently deregulation of Dicer; Exportin 5, mediating pre-miRNA nuclear export, is often mutated and truncated, leaving pre-miRNAs within nucleus [31,32].

3.1. Mutations, SNPs and epigenetics of ncRNAs

Cancer cells have different genetic and epigenetic changes from their normal counterparts and the role of ncRNAs in mediating these differences is beginning to emerge. Specific genetic polymorphisms are associated with the risk of developing several types of cancer [7-9]. Multiple studies have identified small-scale and large-scale mutations and genomic alterations affecting also noncoding regions of the genome. Some of these mutations are structural alterations, rearrangements and chromosomal translocation, amplification, loss of heterozygosity and copy-number variation, nucleotide expansion, and single-nucleotide polymorphisms (SNPs), and they are linking distinct types of mutations in ncRNA genes with diverse

diseases [7]. First, lncRNA have already been implicated in human diseases such as cancer and neurodegeneration [33]. Second, approx. half of miRNA genes are encoded in genomic region prone to cancer-associated rearrangements or in fragile chromosomal sites (amplified, deleted or rearranged) that are often associated with cancer, such as ovarian and breast carcinomas, and melanomas [8,11]. Third, presence of SNPs in miRNAs, where disruption of miRNA target interaction either in the miRNA gene or its target site (3'-UTR mRNA) can lead to complete gain or loss of the miRNA function or target gene thus causing disease [34,35]. In contrast to the miRNA target sites in mRNA transcripts, where the potential of variation is huge, variants identified in miRNA precursor sequences tend to be rarer [36]. The presence of SNPs in *pri-miRNA* or *pre-miRNA* can in addition affect the processing of miRNAs, their expression and/or binding to target mRNA [27,37]. Forth, recent advances in miRNA research have provided evidence of a miRNA association with epigenetic mechanisms activated in diseased human tissues [38]. Heritable changes in gene expression that do not involve coding sequence modification are referred as epigenetics. Gene regulation by ncRNAs was considered as an epigenetic mechanism, but ncRNAs can be regulated by the same mechanism in which they participate [39]. DNA methylation, one of the two major epigenetic mechanisms, leads to gene silencing, and serves as an alternative mechanism of gene inactivation. The aberrant DNA methylation of gene promoters has been shown to result in the inactivation of tumour suppressor genes [40]. For an example, *miR-34* family is a family of tumour suppressors' genes, with *miR-34a* being deregulated by DNA methylation in both epithelial and haematological cancers. *miR-34* is an important component of the p53 tumour suppressor network, and p53 is a predicted target for members of the *miR-34* family. *miR-34a* reinforces the tumour suppressor function of p53, transactivation of *miR-34a* by p53 was also shown to promote apoptosis [11,41-44]. Another example is that *miR-29s* could target two enzymes of methylation process, DNMT3A and DNMT3B [39]. Antisense ncRNAs have been recently showed to be implicated in the silencing of tumour suppressor genes through epigenetic remodelling events [30]. All these miRNAs abnormalities suggest that they play a broad role in cancer pathogenesis.

3.2. Promising role of ncRNAs in cancer: As cancer-subtype classifiers and detection in body fluids

ncRNAs have been recognized as gene-specific regulators. They are similar in activity to a large number of protein transcription factors that are known to be critical in the transformation of cells to a malignant state. Majority of research has been involved in defining the role of miRNAs in cancer; however, lincRNAs have been shown to play role in tumour development by promoting the expression of genes involved in metastasis and angiogenesis [9]. Genome-wide analyses have shown that ncRNAs have distinct signatures specific for a certain cancer type. Importance of combining ncRNAs with other biomarkers for cancer detection and prognosis would improve cancer risk assessment, detection, and prognosis. Thus, there is a need to combine genomic mutations with ncRNA markers to develop marker panels for more accurate risk assessment and early diagnosis [7-9].

Most cancers are diagnosed in advance stages, leading to poor outcome. Intense investigation is going on seeking specific molecular changes that are able to identify patients with early cancer or precursor lesions [1]. Genome-wide expression profiling has examined miR-

NAs in preneoplasia or their usefulness to predict progression from preneoplasia to cancer. Several lines of evidence suggest the potential usefulness of ncRNAs, particularly miRNAs: first, as signature of early events in carcinogenesis and as biomarkers in early cancer detection, second, as differential indicators of benign tumours, preneoplasia, and neoplasia, and third, that miRNAs and perhaps other ncRNAs might be useful in determining which preneoplastic lesions are likely to progress to cancer. Distinguishing benign diseases and certain non-precancerous lesions from precancerous lesions and metastatic tumours would improve patient outcomes, survival, and reduce patient discomfort [45]. Characterization of ncRNAs involved in the development or maintenance of oncogenic states may therefore define ncRNAs as early biomarkers for the emergence of cancer, and could have an impact on the development of tools for disease diagnosis and treatment [30].

miRNAs are believed to be promising potential biomarkers for cancer diagnosis, prognosis and targets for therapy. As potential markers for diagnosis are better classification factors than mRNAs. miRNAs seems to be evolutionarily selected gene regulatory molecules, their expression profiles might therefore be rich in gene regulatory information. Only small percentage of the 16000 genes on the mRNA-expression arrays are regulatory molecules. This difference may be responsible for more efficient microRNA expression arrays in classifying cancer than mRNA-expression arrays [21,45,46]. Some of the key features of miRNAs that make them useful as potential biomarkers can be briefly summarized. First, expression patterns of miRNAs in human cancers appear to be tissue specific. Second, miRNA profiles appear to reflect developmental lineage and differentiation state of the tumours. Third, miRNAs can successfully classify poorly differentiated tumours with high accuracy (~70 %). In contrast, mRNA profiles in the same set of specimens had an accuracy of only 6 %. Therefore, a combination of both miRNA and mRNA profiling data has the potential of enhancing accuracy of tumour classification. Forth, miRNAs can also be profiled and quantitatively measured in formalin-fixed paraffin-embedded tissues. And last, miRNAs are stable in human body fluids of plasma and serum and can be quantitatively measured in microliter quantities of human sera or plasma using qPCR. [45-47].

Highly stable cell-free circulating nucleic acid (cfCNA), both RNA and DNA, has been discovered in the blood, plasma, and urine in humans. Since there is good correlation between tumours and genetic, epigenetic and/or transcriptomic changes and alterations in cfCNA levels, it gives a usefulness of cfCNA as biomarkers for clinical applications. Release of cfCNA in body fluids is probably related to apoptosis and necrosis. Circulating RNAs are stable in serum and plasma in spite of high amounts of RNAase in blood of cancer patients [48]. They are packed in microparticles, of which the most analyzed are in recent years exosomes [3,49,50]. Tumour derived exosomes are small membrane vesicles of endocytic origin released by the tumour and found in peripheral circulation. Several recent reports showed that exosomes could be an important resource of cf-lncRNA/cf-miRNA in serum or plasma [51]. Small size, relative stability and resistance to RNAase degradation make the miRNAs more superior molecular markers than mRNAs [1]. Using non-invasive diagnostic procedures, the extraction and reliable determination of cf-miRNAs, circulating in body fluids like plasma, serum, and others, could serve as circulating tumour biomarkers [52,53].

lncRNAs show greater tissue specificity compared to protein-coding mRNAs, making them attractive in the search of novel diagnostics and/or prognostics cancer biomarkers in body fluid samples. For an example, lncRNA PCA3 was initially identified as over-expressed in prostate tumours relative to benign prostate hyperplasia and normal epithelium. It was later showed that is very specific prostate cancer gene, whose mechanism is not yet identified, but it can be detected in urine samples and has been shown to improve diagnosis of prostate cancer [3].

3.3. ncRNAs can act as both tumour suppressor genes and oncogenes

There are different ways in which miRNAs appear to be involved in cancer: as tumour suppressors, as oncogenes, or as agents involved in affecting genome stability. Below is discussed role of miRNAs acting both, as tumours suppressor and as oncogenes, since are much more investigated in this field than are lncRNAs. Care must be taken in assigning oncogenic or tumour suppressor activity to a miRNA, since miRNA expression patterns are highly specific for cell-type and cellular differentiation status. The same miRNA can function as tumour suppressor in one cell type and as potential oncogene in other cell type. Some of the aberrant miRNA expression observed in tumours may also be a secondary consequence of the loss of normal cellular function that accompanies malignant transformation. Up- or down-regulation of a miRNA in a given tumour type is not obvious a causative role in tumorigenesis [6].

The increased expression of oncogenic miRNAs appears to act in a manner analogous to an oncogene. Over-expression of oncogenic miRNAs are presumed to function by down-regulating the levels of protein product of target tumour suppressor gene or by reduction of tumour suppressor processes, such as apoptosis [2,6,20]. A loss of expression of tumour suppressor miRNA may lead to elevated levels of the protein products of target oncogenes [6], activation of an oncogenic processes, such as proliferation [2,20]. MicroRNAs with anti-proliferative and pro-apoptotic activity are likely to function as tumour suppressors and thus may be under-expressed in cancer cells. Figure 1 represents schematic overview of miRNAs acting as tumour suppressors or oncogenes in comparison to non-cancerous cells.

There should be at least four type of evidence before assigning tumour suppressor function to ncRNAs: (i) data about widespread deregulation in diverse cancer, (ii) gain or loss of function in tumours owing to deletion, amplification or mutation, (iii) direct documentation of tumour suppressing activity using cell line or animal models, (iv) the identification and verification of cancer relevant targets that define mechanisms through which miRNAs participate in oncogenesis [6].

4. ncRNAs as potential therapeutic targets in cancer

4.1. RNAi in therapeutic applications

Using RNAi approaches, ncRNAs may in future serve as therapeutic targets. For ncRNA that is under-expressed and possess tumour suppressor function, re-introduction of the mature

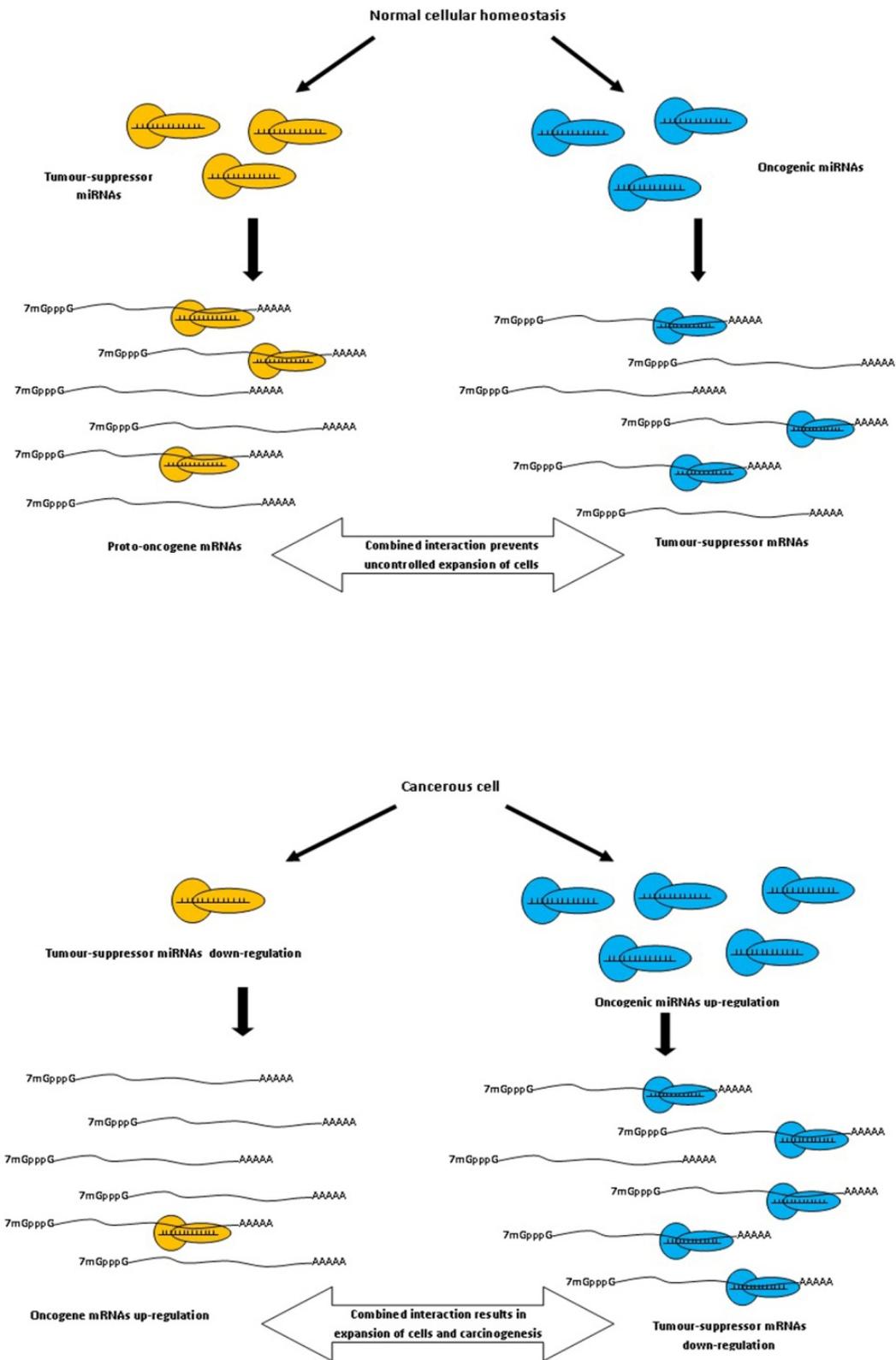


Figure 1. Schematic overview of miRNAs acting as tumour suppressors or oncogenes.

ncRNA into the affected tissue would restore the regulation of the target gene. By contrast, over-expressed ncRNA with oncogenic function could be down-regulated by reducing mature ncRNA level by its direct targeting [54].

Due to the interferon response it is difficult to introduce long dsRNAs into mammalian cells, however, the use of RNAi as a therapeutic approach has been successfully used. Among the first applications to reach clinical trials were in the treatment of macular degeneration and respiratory syncytial virus infection, reversal of induced liver failure in mouse models, antiviral therapies, neurodegenerative diseases, and cancer. Cancer was treated by silencing up-regulated genes in tumour cells or genes involved in cell division. A key area of research in the use of RNAi for clinical applications is the development of a safe delivery method, which to date has involved mainly viral (lentivirus, adenovirus, adeno-associated virus) and non-viral (nanoparticles, aptamers, stable nucleic-acid-lipid particle, e.g.) vector systems similar to those suggested for gene therapy [55].

4.2. ncRNAs with tumour suppressor function as therapeutic targets

Replenishing small RNAs/miRNAs

Pharmacological manipulation of miRNAs is still in its infancy; however, the correlation between the expression of miRNAs and their effects on target oncogenes, on tumorigenesis, and on the proliferation of cancer cells has gained experimental support. miRNAs are small molecules, making their *in vivo* delivery feasible. It has been shown that miRNAs can be delivered systematically, and can reduce invasion, proliferation and growth as well as induce radio-sensitivity and resistance. miRNAs may therefore serve as therapeutic targets in the future.

For miRNA that is under-expressed, re-introduction of the mature miRNA into the affected tissue would restore regulation of the target gene. For this purpose, artificial miRNA (miRNA-mimic) have been developed to enhance the expression of beneficial miRNAs or the introduction of short hairpin duplex, similar to *pre-miRNA*, into the cell. This suggests that individual miRNAs are potential therapeutic agents, provided that their expression or delivery can be targeted to appropriate tissue. Most of the developed protocols have used local administration in easily accessible tissue; systemic delivery has also give some promising results; the major challenge remains tissue and cell-type specific targeting [56].

miRNA mimic can only last a couple of days and the long term biological effects were not observed very effectively. To overcome this, the cells were infected with a lentivirus that expressed mature miRNAs. This generated stable cell expressing miRNAs. miRNA mimics and lentiviral miRNAs showed great potential in restoring tumour suppressor miRNAs. However, viral and non-viral delivery systems have been developed. Viral vector-directed methods show high gene transfer efficiency, but have some limitations. However, non-viral gene transfer vectors have been also developed: cationic liposome mediated gene transfer system, lipoplexes, neutral lipid emulsion, etc. [57].

Expression of miRNA-mimic would simultaneously suppress many gene targets. miRNAs-mimic would be useful in conjunction with standard chemotherapy or radiotherapy,

by influencing drug resistance or enhancing responsiveness to therapy. Current limitation is need for improvement of efficiency of delivery to target tissue, for systemic drug administration, potential inhibition of non-target genes (“off-target effect”), redundancy among miRNAs efficacy, potential toxicity and immunogenic responses. However, studies introducing miRNAs strategies to inhibit cancer propagation in animal models are showing promising results [32].

Examples for miRNA

Therapeutic delivery to animal models was demonstrated using miRNA-mimics of the tumour suppressor miRNAs, *miR-34a* and *let-7a*, both of which are often down-regulated or lost in lung cancer. It has been shown that re-introduction of *let-7* directly represses cancer growth in the lung [58] and that development of chemically synthesized therapeutic *miR-34a* and lipid-based delivery vehicle block tumour growth in mouse models of non-small-cell lung cancer (NSCLC) [59]. Systemic treatment of these mice led to significant decrease in tumour burden. Mice treated with *miR-34a* displayed a 60 % reduction in tumour area compared to mice treated with a miRNA control. Similar results were obtained with the *let-7* mimic [60].

Targeting lncRNAs

Successful inhibition of lncRNAs seems to be more difficult than inhibition of miRNAs. Our growing knowledge of other ncRNAs might exploit in future to develop new therapeutic strategies not only against cancer, but also for other diseased states. The findings regarding lncRNA and Alzheimer disease are attracting the attention of pharmaceutical and biotechnology industries [8]. Therapy using small RNAs that targets ncRNA transcripts, such as eRNAs or PARs, may represent a new way to treat disease conditions caused by epigenetic changes [9].

Targeting both, lncRNAs and miRNAs

Another possible approach for manipulation of ncRNAs level may also be by altering DNA methylation. As mentioned above, DNA methylation is a crucial mechanism associated with epigenetic regulation. It has been shown that in cancer cells treated with DNA demethylating agent reactivation of certain miRNAs occurs [40]. ncRNAs mediated therapy may also be useful in combination with DNA methyltransferase inhibitors that are other way toxic [39].

5. ncRNAs as tumour suppressor in different types of cancers

5.1. miRNAs as tumour suppressors

In the following section, down-regulated miRNAs will be describe and miRNAs with suggested tumours suppressive roles in different types of cancer. However, down-regulation does not necessary mean that miRNA is tumours uppressor.

Hematological cancers

Leukaemia. Chronic lymphocytic leukaemia (CLL) is characterized by overexpression of the protein Bcl-2 in B cells and represents the most common human leukaemia. In less than 5 %

of cases, over-expression of Bcl-2 is due to a translocation of the Bcl-2 gene, whereas for the majority of CLL cases no explanation for the deregulation of Bcl-2 has been reported. It has been demonstrated that mutations in genomic regions containing miRNAs were associated with disease progression in a number of CLL patients. In this type of cancer, *miR-15* and *miR-16* expression is often reduced and indeed, one of the first associations between miRNAs and cancer development was observed for *miR-15* and *miR-16* in CLL [46]. Both miRNAs are located in a 30 kb region on chromosome 13 that had been found deleted in more than half of B cell CLL (chromosome 13q14 deletion) [8,46], and *miR-15a* and *miR-16-1* have been shown to be deleted or translocated in approx. 65 % of CLL patients [2,11]. Several papers indicate that miRNA regulates cell growth and apoptosis. Indeed, over-expression of *miR-15* and *miR-16* directly inhibit anti-apoptotic Bcl-2, a key player in many types of human cancers, and thus activate apoptotic processes [2,11]. However, it was further demonstrated that other mutations in miRNA genes are frequent in CLL; many mutations were located in the flanking sequence of *pre-miRNA*, thus cell culture assay indicated that a point mutation of the *miR-16-1* precursor abolishes expression of mature *miR-16* [21]. Few other miRNAs were also recognized as tumour suppressors in CLL. *miR-29a* and *miR-29b* are associated with fragile site FRA7H that is not associated with any known tumour suppressor gene. Over-expression of *miR-29b* may target TCL1 and reduces anti-apoptotic Mcl-1 protein in CLL patients [11]. Another well-known tumour suppressor was analysed. Low expression of *miR-34a* in CLL was found to be associated with p53 inactivation, impaired DNA damage response, apoptosis resistance and chemotherapy-refractory disease irrespective to p53 mutation (cases with CLL with p53 mutation are resistant to chemotherapy). It was latter showed that *miR-34a* is induced by p53. In another type of leukaemia, particularly acute myeloid leukaemia, an inverse correlation between *miR-34b* and CREB expression has been observed. After restoring expression of *miR-34b*, cell cycle abnormalities, reduces growth and altered CREB expression has been observed, suggesting tumour suppressor potential of this miRNA [47].

Lymphoma.miR-142 gene was found at the junction of the t(8;17) translocation, which may contribute to the progression of an indolent lymphoma into aggressive B-cell leukaemia [21].

Breast cancer

Breast cancer is one of the most important cancers in adult females. *miR-125b*, *miR-145*, *miR-21* and *miR-155* were significantly reduced in breast cancer tissue and this expression was correlated with specific breast cancer pathologic features, such as tumour stage, proliferation, oestrogen and progesterone receptor expression, and vascular invasion. Some of these miRNAs act as oncogenes (e.g. *miR-21*) in many cancer types, so it is suggested that some miRNAs act as tumour suppressors in one cancer type and as oncogenes in another [2]. *miR-125b-1* is located on a fragile site on chromosome 11q24, which is deleted in a subset of patients with breast cancer [61]. Down-regulation of *mir-221* in breast cancers was detected, whereas germ line mutation in mature *miR-125a* is highly associated with breast cancer tumorigenesis, suggesting its tumour suppressor role. *miR-125a* is also down-regulated in human breast cancer and when over-expressed post-transcriptionally regulates CYP24 resulting in an anti-proliferative effect. Ectopic expression of *miR-30e* suppresses cell growth in breast cancer, probably through targeting Ubc9 [47]. *miR-17-5p* was down-regulated in breast

cancer cells, and enhanced expression decreased tumour cell proliferation [11]. Cyclin D1 has been identified as a direct target for *miR-17/20* that functions to suppress proliferation of breast cancer cells. Additional miRNAs have also been shown to be down-regulated and have tumour suppressor function in other types of cancer: *let-7*, *miR-145*, *miR-34a*, *miR-214*, and *miR-205*. MicroRNAs, *miR-31*, *miR-126*, *miR-146a/b*, *miR-206* and *miR-335* have been shown as anti-metastatic miRNAs [62,63].

Colorectal Cancer (CRC)

Colorectal cancer is the third most commonly diagnosed cancer in the world, but it is more common in developed countries. Also in colorectal neoplasia miRNAs expression is associated to the tumour formation. Reduced expression of *miR-143* and *miR-145* have been shown to be a frequent feature of colorectal tumours (adenomatous and cancer stage) when compared to normal mucosa [2,6,64]. A tumour suppressive role of *miR-143* has been elucidated in the epigenetic aberration of CRC with DNMT3A as a target. Restoration of *miR-143* expression in CRC decreases tumour cell growth and down-regulates DNMT3A expression [47]. *let-7* has been implicated in development of colon cancers and progression of colorectal cancers; together with *miR-143* and *miR-18a* was observed to be down-regulated and target KRAS [11, 64]. *miR-145* has been proposed as a tumour suppressor and it has been shown that target IRS-1, and when over-expressed it dramatically inhibits the growth of colon cancer cells. A ubiquitous loss of *miR-126* expression in colon cancer lines was observed and its reconstitution resulted in a significant growth reduction. Also, in a panel of matched normal colon and primary colon tumours, each of the tumours demonstrated *miR-126* down-regulation [64]. Down-regulation of *miR-200* family is a hallmark of EMT as well as up-regulation of ZEB1 transcription factor, and it was shown that in colorectal cells ZEB1 directly suppress transcription of *miR-141* and *miR-200c* [57]. It was found that *miR-192* and *miR-215* was down-regulated in CRC, and their anti-proliferative effect was identified in CRC cell lines. It was further defined that both are regulated by p53 and that their targets are a number of transcripts that regulate cell cycle checkpoints [57]. An inverse correlation between COX-2 and *miR-101* was reported in CRC cell lines, and this was further confirmed in colon cancer tissue and liver metastases derived from CRC patients. *miR-16*, *miR-125b*, *miR-31*, *miR-133b*, and *miR-96* were along with already mentioned miRNAs showed to be down-regulated in colorectal cancer [1].

Aberrant DNA methylation may further induce silencing of specific miRNAs in CRC. While methylation of *miR-129* and *miR-137* CpG islands is frequently observed in CRC, is methylation of *miR-9-1* associated with the presence of lymph node metastasis and expression of *miR-9* in CRC inversely correlated with the methylation of its promoter regions [47]. In human colon cancer cell lines, *miR-34a* was showed to participate in the apoptotic program triggered by p53 activation and loss of *miR-34a* expression occurs frequently in cancer cells. p53 directly binds to the genomic region defined as the *miR-34a* promoter with consequent *miR-34a* targeting genes of cell cycle, DNA repair, mitotic checkpoint, DNA integrity checkpoint, cell proliferation, and angiogenesis. Among the down-regulated targets of *miR-34* family were well-characterized p53 targets, such as CDK4/6, cyclin E2, E2F5, BIRC3 and Bcl-2. These effects were nearly identical irrespective of whether *miR-34a*, *miR-34b* or *miR-34c* was introduced into cell lines. Another target was identified for *miR-34a*, SIRT1, negative regulator of apoptosis.

miR-34a promoter hyper-methylation was observed in 3 of 23 cases of colon cancer, *miR-34b/c* were found to be epigenetically silenced in 9 of 9 cell lines and in 101 of 111 primary CRC tumours [42,64].

Gastric cancer

Gastric cancer is the fourth most common cancer and the second leading cause of cancer death in the world. It was reported that loss of Ago2, which leads to premature stopping of miRNAs biogenesis and general deregulation of miRNAs expression, was observed in 40 % of human gastric cancer patients with high microsatellite instability. A number of miRNAs were reported to be down-regulated. Among these, *miR-141* was significantly low expressed in 80 % of primary gastric carcinoma compared to non-cancer adjacent tissue. It targets FGFR2 and its down-regulation means proliferative potential and poor differentiation of gastric cancer cells [65]. It appears that down-regulation of *miR-451* is related to the worse prognosis of the gastric cancer patients. Over-expression of *miR-451* in gastric cancer cells regulates the oncogene MIF production, reduces cell proliferation and increases sensitivity to radiotherapy [47]. *miR-101* was down-regulated in gastric cancer cells, its targets are: EZH2, Cox-2, Mcl-1, and Fos [65]. Other potential tumour suppressor miRNAs in gastric cancer are: *miR-181b/c* and *miR-432AS*, and for *miR-181* it was proposed that modulate expression of Bcl-2. Low level or loss of expression in gastric cancer also showed *let-7a*, *miR-486*, and *miR-449*. However, a proposed role for *miR-107* and *miR-126* is controversial in gastric cancer, either tumour-suppressive or oncogenic [66].

An epigenetic silencing of *miR-512-5p* was observed in gastric cancer cells. As its target is was shown anti-apoptotic protein Mcl-1 and after epigenetic treatment (demethylation), it results in apoptosis of gastric cancer cells [65].

The association between genetic polymorphism of *miR-196a-2* and risk of gastric cancer has been identified; it was found that the variant homozygous genotype of *miR-196a-2* was associated with significantly increased risk of gastric cancer [65].

Pancreatic cancer

Pancreatic cancer is the eighth most common cause of cancer-related deaths worldwide and it has a poor prognosis for all stages. It is usually diagnosed at advent stages, therefore it is an urgent need to find some specific biomarkers and key components of carcinogenesis. Several miRNAs were reported to suppress metastasis. In pancreatic cancer cell lines, *miR-146a* was decreased compared to normal ductal epithelial cell line, and its ectopic expression inhibited invasive capacity of pancreatic cancer cell lines. *miR-96* is believed to be a potential tumour suppressor through targeting KRAS. It is significantly down-regulated in pancreatic cancer, its ectopic expression induces apoptosis, inhibits cell proliferation, migration and invasion. In human clinical samples there is observed inverse correlation between *miR-96* and KRAS. Further, ectopic expression of *miR-520h* has inhibitory effect on pancreatic cancer cell migration and invasion, *miR-20a*, with metastasis-suppressing effect, is reduced in pancreatic cancer and its cell lines. [67]. In pancreatic ductal carcinoma, *miR-345*, *miR-139*, and *miR-142-p* were the most down-regulated miRNAs in tumour tissue compared to normal tissue [1]. Other potential

tumour suppressor miRNAs involved in pancreatic cancer are: *miR-100*, *miR-181a*, and *miR-15b*, but are as well as *miR-200* family up-regulated [67].

miR-200 family, potential tumour suppressors, is up-regulated in pancreatic cancer. Low expression of *miR-200* family genes and higher expression of their target is common in different cancers. However, most pancreatic cancer cell investigations showed hypo-methylation of *miR-200a/b* and its over-expression, in contrary its targets are hyper-methylated, suggesting that this pathway is not involved in metastases in most pancreatic cancer [67]

Hepatocellular Carcinoma (HCC)

Primary liver cancer mainly refers to HCC, which is one of the most common malignant tumours in liver and accounts for 85-90 % of primary liver cancers. Cyclins D2 and E2 were validated as direct targets for *miR-26a*, which exhibit reduced expression in HCC [68]. In another early study on animal models, down-regulation of number of miRNAs has been detected, including known tumour suppressor miRNAs, such as: *miR-15/16*, *miR-34a*, *miR-150* and *miR-195* [69]. *miR-122*, which represents 70 % of all liver miRNAs, was found to be frequently down-regulated in HCCs. Loss of *miR-122* expression in tumour cells segregates with specific gene expression profiles linking to HCC progression. *miR-122* is specifically repressed in a subset of primary HCCs that are characterized by poor prognosis and is therefore suggested as tumour suppressor miRNA [47,68]. As one of *miR-122* targets, cyclin G1 was identified, through its regulation *miR-122* influences p53 protein stability and transcriptional activity. Two other *miR-122* targets, which promote tumorigenesis, are SRF and IGF1R. The cellular mRNAs and protein levels of Bcl-w were also repressed by *miR-122*. Other pro-apoptotic functions were assigned for *let-7* through targeting Bcl-xL, for *miR-101* through targeting Mcl-1, and for *miR-29* through targeting Mcl-1 and Bcl2. *miR-195* was significantly reduced in HCC tissues and cell lines, it suppress tumorigenicity through targeting cyclin D1, CDK6, and E2F3. CDK6 was showed to be also target for *miR-124*, which is silenced through CpG methylation in HCC; *miR-124* in addition targets vimentin, SET, and MYND. *Let-7g* inhibits the proliferation of HCC by down-regulating c-Myc. Methylation of *miR-1* in HCC results in enhanced tumour cell growth, probably through release of its oncogenic targets c-Met, FoxP1, HDAC4. *miR-223* targets Stahmin 1 and is down-regulated in HCC whereas *miR-375* inhibits the proliferation and invasion of HCC cells by targeting Hippo-signalling effectors YAP [68,70]. Research on expression profiling in HCC and adjunct non-tumour tissue defined that *miR-199a**, *miR-195*, *miR-199a*, *miR-200a*, and *miR-125a* were also under-expressed in HCC tissue [2]. Anti-metastatic functions were showed for *miR-122* by targeting desintegrin and metalloprotease, and *let-7g* by targeting type I collagen A2. c-Met is target for *miR-1*, *miR-34a*, *miR-23b* and *miR-199a-3p*, and all of these miRNAs are down-regulated in HCC [68,70].

Lung cancer

Lung cancer is one of the most common cancers of adults and is also leading cause of cancer-related deaths in many economically developed countries [2]. *Let-7* family is a family of miRNAs, whose genes map to different chromosome regions that are frequently deleted in lung cancer [71]. Significantly worse survival was observed among patients with low expres-

sion of *let-7a-2* compared to those with opposite expression pattern, independent of disease stage [2]. Over-expression of *let-7* in lung adenocarcinoma cell lines inhibited cancer cell growth and reduced cell cycle progression. These findings reflect that *let-7* mediates tumour suppressive function [2,45]. It has been shown that *let-7* regulates the expression of several oncogenes, RAS, MYC, HMGA2, and cell-cycle progression regulators, CDC25, CDK6, cyclin D2. *let-7* is down regulated in lung tumours and its expression anti-correlated with that of RAS relative to the normal lung tissue [46,71]. In animal models, ectopic *let-7g* expression reduces tumour burden and intranasal administration repress lung adenocarcinoma. A SNP in *let-7* complementary site 6 in 3'-UTR of its target KRAS is significantly associated with increased risk for NSCLC among moderate smokers [71]. The *let-7* family was subsequently found to be deregulated in a large number of tumour types [46].

Mutations in EGFR gene are more frequent in NSCLC patients who never smoked tobacco; a significant down-regulation of *miR-145* has been demonstrated in the cancer tissues of these patients. Restoration of this miRNA can inhibit cancer cell growth in EGFR mutant lung AD [47,71]. *miR-128b* has been also showed as direct regulator of EGFR, whereas *miR-128b* loss-of-heterozigosity is frequently found in NSCLC. *miR-7* is frequently down-regulated in lung cancer, it suppress EGFR and Raf1, it attenuates activation of Akt and ERK, suggesting that is negative regulator of EGFR pathway [71].

It was proposed that *miR-140* regulates PDGF in lung cancer development, but this has not yet been thoroughly investigated. *miR-29* is down-regulated in lung cancers, its targets are Mcl-1, DNMT3A and DNMT3B, suggesting that it is pro-apoptotic and that it has role in regulating epigenetic DNA methylation. Further, in lung cancer cells induction of *miR-34* results in apoptosis; miRNA profiling revealed that *miR-34a/b/c* are directly correlated with expression of p53. Decreased expression of *miR-126* and increased expression of VEGFA was found in various lung cancer cell lines. Introduction of *miR-126* down-regulates VEGFA, inhibits growth, and reduces average tumour weight. Mouse model of lung adenocarcinoma showed that *miR-200* family possess anti-metastatic abilities [5]. *miR-125b-1* is located on a fragile site on chromosome 11q24 which is deleted in a subset of patients with lung cancer [61].

Human brain cancer

The phrase "brain tumours" describes an inhomogeneous collection of various tumours of the brain, which represents primary tumours of nervous central system or metastases. Glioblastomas (belongs to family of gliomas) are the most frequent occurrence and malignant form of primary brain tumors in contrary to medulloblastomas, which have a better prognosis [2,11]. Several articles have described the effects of ectopic miRNA modulation on medulloblastoma cell proliferation and growth.

Rescued expression of *miR-9* and *miR-125a* were shown to promote medulloblastoma cell growth arrest and apoptosis by targeting TrkC, whereas *miR-29* has been shown to be down-regulated in neuroblastoma and brain tumour [47,72]. Further, *miR-34a* induces apoptosis in neuroblastoma cells, possibly by targeting the transcription factor E2F3 [11]. Transient transfection in medullablastoma cells with *miR-34a* strongly inhibited cell proliferation, cell cycle progression, cell survival and cell invasion. *miR-34a* was shown to inhibit c-Met, Notch-1,

Notch-2 and CDK6. Ectopic up-regulation of *miR-124* was shown to inhibit cell proliferation, and it was demonstrated that *miR-124* target and regulates CDK6. *miR-125b*, *miR-324-5p*, and *miR-326* over-expression inhibit medulloblastoma cell growth by targeting Hedgehog signaling pathway. *miR-128* also inhibits growth by targeting Bmi-1 oncogene. Another miRNA, *miR-199b-5p* negatively regulates proliferation and cell growth [72].

In contrary to medulloblastomas, gliomas are the most common and deadly primary human brain tumours, and its subtype glioblastomas are highly invasive, very aggressive, and one of the most incurable [2]. *miR-181a*, *miR-181b* and *miR-181c* were originally identified as down-regulated in glioblastoma cells and tumours when compared to normal brain controls. *miR-181a* and to a greater extent *miR-181b* were subsequently described as tumour suppressors that inhibit growth and induce apoptosis of glioma cells. *miR-181a* over-expression down-regulates Bcl-2. Several other miRNAs have been implicated in glioma malignancy as tumour suppressors. *miR-15b* was suggested to target CCNE1, the gene encoding cyclin E1, however, a direct link between CCNE1 down-regulation by *miR-15* and cell cycle regulation was not demonstrated. *miR-146b* was shown to inhibit glioma cell migration and invasion, and was identified as one of miRNAs that is significantly deregulated in human glioblastoma tissue. *miR-146b* over-expression or knock-down did not affect the growth of human glioblastoma cell line, while it significantly reduced the migration and invasion of one glioblastoma cell line. MMP16 was identified as one of the downstream targets of *miR-146b*. *miR-125b* was shown to induce cell cycle arrest and inhibits CDK6 and CDC25A expression in glioma cell lines. However, another study suggested oncogene function for *miR-125b*. *miR-153* decrease cell proliferation and increased apoptosis (pro-apoptotic miRNA), it inhibited Bcl-2 and Mcl-1. *miR-17* and *miR-184* were identified as two miRNAs with reduced expression in higher grades of glioblastomas. Their over-expression inhibited viability, proliferation, invasion and decreased expression of AKT 2 and several other genes. *let-7* over-expression effect was investigated in glioma cells, its transfection reduced expression of RAS oncogenes, proliferation in vitro, migration of the cells, and reduced the size of tumours generated [73].

Head and Neck Squamous Cell Carcinoma (HNSCC)

Head and neck tumours are a heterogenous group with different behaviour at the various sites arising from anatomical factors, cell-type variation, and differences in exposure to risk factors including tobacco, alcohol, and viruses [74]. Head and neck squamous cell carcinoma is represented by epithelial cancers of the oral cavity, pharynx, nasal cavity, paranasal sinuses, salivary glands and larynx [75]. Studies were made in expression profiling regarding different sites of head and neck tumours, tongue, tonsil, larynx, hypopharynx, nasopharynx, saliva, oral cavity, salivary gland and animal models. Several miRNAs were identified as down-regulated, and for some of their target genes were validated [74].

Low expression of *miR-205* is significantly associated with local-regional recurrence independent of disease severity at diagnosis and treatment. Combined low expression of *let-7d* and *miR-205* is significantly associated with poor survival. In nasopharyngeal carcinoma down-regulation of *miR-34* family, *miR-145* and *miR-143* was also observed [47]. Tumour suppressive role in HNSCC has been suggested for *let-7* family, *miR-125a/b*, *miR-200*, *miR-133a/b*, and *miR-100* [75]. General down-regulation of *miR-1*, *miR-133a* and *let-7b* was also observed [74].

Reduced expression for majority of members of the *let-7* family (except *let-7i*) was observed in HNSCC. KRAS and HMGA2 have been characterized as targets for *let-7* [75]. Notable among down-regulated was also *miR-98*. However, another group identified *miR-98* as another regulator of an oncogene HMGA2 [74]. A possible molecular mechanism of *miR-125a/b* down-regulation might be through targeting ERBB2, since its higher level of expression was observed in oral SCC. Other target were also suggested for *miR-125*, namely KLF13, CXCL11 and FOXA1 [74,75]. Down-regulation of *miR-133a/b* in primary HNSCC may further contribute to increased cell proliferation and decreased apoptosis. PKM2 has been validated as cellular target for both, *miR-133a* and *miR-133b*, and increased expression of PKM2 has been associated with cancer progression. Finally, *miR-100* has been observed at suppressed levels in primary HNSCC and derived cell lines. A few of its targets are known, namely FGFR1, MMP13, ID1, FGFR3, EGR2. The exact role has to be investigated yet, but suggestion has been made that down-regulation of *miR-100* means higher rate of cell proliferations.

Deregulation of miRNAs in cancer can occur through epigenetic changes (promoter CpG island hyper-methylation in the case of *miR-200* family) [8]. Suppressed *miR-200a* was detected in primary oral SCC. Members of *miR-200* family inhibit EMT by directly targeting ZEB1/ZEB2, suppressed levels of *miR-200a* may promote EMT. At last, it was observed that *miR-137* and *miR-193a* could be also silenced via hyper-methylation, CDK6 and E2F6 has been suggested as their major targets. It was also shown that *miR-137* hyper-methylation is associated with poorer average survival [74,75].

Urological tumours

Renal cell carcinoma (RCC). The VHL tumour suppressor signalling pathway is the most important deregulated pathway in clear cell RCC, the dominant subtype of kidney cancers. The VHL gene can be spontaneously deleted or hyper-methylated. The regulation of the VHL pathway by miRNAs has not been well studied in RCC. The interactions have been proposed but a direct relation between miRNAs and VHL or HIF1A were not proven. A subset of miRNAs has been identified as regulated by VHL pathway, 3 miRNAs were up-regulated and 6 were down-regulated. The second commonly deregulated pathway is VEGF signalling pathway, which is transcriptionally regulated by HIF (after hypoxia) or due to loss of VHL. Interaction between miRNAs and VEGF has not been well studied in RC, and only *miR-29b* has been shown to indirectly regulate VEGF. However, strong inverse correlation between *miR-200* family and VEGFA has been observed with suggestion that VEGFA is direct target of these miRNAs [76]. Down-regulation of *miR-141* was found in malignant compared to matched non-malignant tissue samples [47].

Bladder cancer. Mutation or over-expression of the FGFR3 gene occurs in approx. 80 % of all patients with low-grade non-invasive urothelial carcinomas. Some well-established deregulated miRNAs in bladder cancer are predicted to target FGFR3, and regulation of FGFR3 by *miR-99* and *miR-100* of four predicted miRNAs has been experimentally validated. Family *miR-200* is associated with an epithelial phenotype; its ectopic expression in bladder cancer cell lines induces up-regulation of epithelial and down-regulation of mesenchymal markers. Up-regulation of *miR-143* is accompanied by down-regulation of RAS [76]. Transfection of bladder cancer cell lines with *pre-miR-129* exerts significant growth

inhibition and induces cell death. *mir-129* is shown to target GALNT1 and SOX4. Transfection of *miR-30-3p*, *miR-133a* and *miR-199a** results in decrease of tumour cell growth in bladder cancer. *miR-101* inhibits cell proliferation in bladder transitional cell carcinoma by targeting EZH2 and altering global chromatin structure. Down-regulation of *miR-145* in bladder cancer has been also observed [47].

Prostate cancer. A major signal transduction pathway in prostate cancer is PI3K/Akt signalling pathway that is hyper-activated in approx. 30-50 % of prostate cancer. Many of the predicted miRNAs that are predicted to target proteins of this pathway are differentially regulated in prostate cancer. Second pathway is androgen receptor (AR) pathway. *miR-125b* is an androgen-sensitive miRNA, which has been shown to regulate apoptosis through inhibition of BAK1. *miR-101* has been shown to be up-regulated in human prostate cancer and it seems that through inhibition of EZH2 reduce invasion and induce morphological changes in prostate cancer cells [76]. *miR-221* is found to be progressively down-regulated in aggressive forms of prostate cancer. Down-regulation of *miR-221* is linked to cancer progression and recurrence in a high risk prostate cohort. Progressive miR-221 down-regulation also hallmarks metastasis [47,76]. *miR-499a* has been shown to be down-regulated in prostate cancer tissue. Its introduction into prostate cancer cells results in cell cycle arrest and apoptosis, where it regulates cell growth and viability in part by repressing the expression of HDAC1 [47]. *miR-15a-miR-16-1* cluster, located at chromosome 13q14, is deleted in most cases of prostate cancer [21].

Melanomas

Melanoma is the most aggressive type of skin cancer, and it is resistant to therapy in its advanced stages [77]. Abnormalities in several signal transduction pathways, which are important for normal melanocyte development, only partly explain molecular mechanism directly linking UV radiation to the development of melanoma. miRNAs are emerging as important causal factors to melanoma initiation and progression [78]. In 45 primary cultured melanoma cell lines, there was observed that many genomic loci containing miRNAs are frequently affected (85.9 %) by copy number abnormalities. For an example, copy number losses of the region containing *miR-218-1* and SLIT2 were shown in 33 % of all investigated melanoma lines [79]. Proteins involved in miRNA biogenesis, Drosha, Dicer and Ago, are over-expressed in melanoma [32].

However, deregulation of miRNAs expression is not always explained. *let-7* plays a role in melanoma development and progression, its targets are many cancer-promoting molecules, such as NRAS, Raf, c-myc, cyclin D1/D3, and CDK4 [77]. Analyzing 10 melanocytic nevi and 10 primary melanomas, it was revealed that five members of the *let-7* family were significantly down-regulated in melanoma [80]. Over-expression of *let-7b* leads to inhibition of cell cycle progression and inhibition of its targets (cyclin D1/D3/A and CDK4) [77]. A direct interaction of *let-7b* with the cyclin D1 3'-UTR was showed. *Let-7a* was thus demonstrated to regulate expression of integrin beta3 and RAS oncogene, which is highly related to melanoma progression.

Another family, *miR-34*, has tumour suppressive role. Expression of *miR-34a* is silenced due to an aberrant CpG methylation in 43.2 % of melanoma cell lines and in 62.5 % of primary

melanoma samples. The tumour suppressive function of *miR-34a* has not yet been investigated, however, there was shown a reduced expression of *miR-34b/c* and *miR-199a** and it was proposed that their target is MET oncogene [80]. Another miRNA with potentially tumour suppressor role is embedded in CpG island and epigenetically regulated in melanoma, this is *miR-370* [79].

miR-203 has also an important tumours suppressor role in a lot of cancers, and it is often lost due to deletion or due to promoter CpG hyper-methylation. *miR-203* target p63, and their relationship might be relevant also in melanoma, since it is known that *miR-203* functions as switch between epidermal proliferation and differentiation [81].

Gynaecological tumours

miR-125b-1 is located on a fragile site on chromosome 11q24 which is deleted in a subset of patients with ovarian and cervical cancer [61].

Cervical cancer. Cervical cancer aetiology is strongly linked to HPV infection, and involvement of virus protein E6 and E7 in pathogenesis is well established. The exact pathway from infection to tumorigenesis has not been elucidated yet. However, down-regulation was observed for: *let-7b/c*, *miR-23b*, *miR-196b*, *miR-143*, and *miR-145*. *miR-143* and *miR-145* were equally down-regulated in all cell lines of cervical cancer (HPV infected and HPV not infected), whereas *miR-218* was the unique miRNA down regulated only in HPV-16 and HPV-18 positive cell lines. Down-regulation of *miR-214* is related to the ability of this miRNA to inhibit HeLa cells proliferation through targeting MEK3 and JNK1 transcripts. HPV protein E6 induces destabilization of p53, down-regulation of *miR-34a* and increased proliferation of pre-malignant HPV infected cervical cancer cell lines [82].

Endometrial cancer. Reciprocal association between down-regulation of *miR-192-2* and SOX4 expression was determined; it was further established that restoration of *miR-192-2* induced a decrease in SOX4 expression and this resulted in diminished cell proliferation. Decreased expression for *miR-152* and *miR-101* was found to consist of an independent risk factor for disease free survival. Restoration of those miRNAs by transfection in cell lines lead to diminished cell proliferation. Down-regulation of *miR-101* was correlated with strong positive immunoreactivity of COX2, which was previously shown to be associated with worse prognosis. To date, no data are available for relationship between miRNAs and oestrogen response in endometrial cancer [82].

Ovarian cancer. Inconsistencies are observed between results in ovarian cancer studies for well-known tumour suppressors. These could be due to the differences in study populations and methodologies used, due to the choice of control group and type of control. For instance, the number of studies used as control cell lines and another number of studies used whole normal ovaries. The existence of significant discrepancies in expression profiles of certain miRNAs indicate the need of further and more in-depth research that would establish those results [82].

5.2. lncRNAs as tumour suppressors

lncRNAs are known to mediate epigenetic modifications of DNA by recruiting chromatin complexes to specific loci [8]. Only a handful of lncRNAs have been characterized, and their involvement in control of gene expression [3]. We therefore presented four lncRNAs with proposed tumour suppressor function in cancer.

MEG3

MEG3, located in chromosome 14q32, is maternally expressed imprinted gene, which represents lncRNA, but also hosted miRNAs and snoRNAs. It plays role in cell proliferation, and its expression is under epigenetic control. *MEG3* and its hosted miRNAs and snoRNA could represent a tumour suppressor gene, since aberrant CpG methylation (promoter hypermethylation, and hyper-methylation of the intergenic region) has been observed in several types of cancer, as well as their gene copy number loss [9].

MEG3 ncRNA might modulate binding of p53 on the promoter of its target genes [9]. It was later verified that *MEG3* was associated with p53 and that this association was required for p53 activation, further suggesting tumour suppressor role for *MEG3*. It was demonstrated that *MEG3* expression is markedly decreased in glioma tissues compared to adjunct normal tissues. Ectopic expression of *MEG3* inhibited cell proliferation and promoted cell apoptosis in glioma cell lines [83]. Growth inhibition is partially due to apoptosis induced by *MEG3*, which induces accumulation of p53, stimulates transcription from p53-dependent promoter and regulates p53 target gene expression. Loss or significantly reduction of *MEG3* expression has been further found in other cancer cell lines examined, bladder, bone marrow, breast, cervix, colon, liver, lung, meninges, and prostate, as well as in other primary tumours, neuroblastoma, hepatocellular carcinoma, and meningioma. It has been suggested that DNA methylation plays a major role in silencing the *MEG3* gene in tumours [84].

GAS5

lncRNA *GAS5* is highly expressed in cells that have arrested growth and can sensitize a cell to apoptosis by regulating activity of glucocorticoids in response to nutrient starvation. It has been linked with breast cancer. *GAS5* transcript levels are significantly reduced compared to un-affected normal breast epithelia, suggesting that could act as tumour suppressor. *GAS5* maintain sufficient caspase activity to activate appropriate apoptotic response in diseased cells. Chromosomal translocation affecting 1q25 locus that contains the *GAS5* gene has been detected in melanoma, B-cell lymphoma, prostate and breast cancer [7,85]. *GAS5* regulates expression of a critical subset of genes with tumour suppressive consequences [4].

LincRNA-21

LincRNA-p21 is required for the global repression of genes that interfere with p53 function regulating cellular apoptosis; it physically interacts with a protein hnRNP-K, allows its localization to promoters of genes that need to be repressed in a p53-dependent manner [4]. In response to DNA damage, lncRNAs are induced by the p53 tumour suppressor pathway. *lincRNA-p21* plays an important role in cellular response to apoptotic signal, it is induced by p53 and act as an inhibitor of the p53-dependent transcriptional response by repressing the

transcription of genes that interfere with apoptosis (guidance of hnRNP-K to the promoters of genes repressed by p53). *LincRNA-p21* has not been directly associated with disease yet, but loss of function of *lincRNA-p21* might be involved in cancer initiation since functions to trigger cell death through the induction of apoptosis program [7,9,85].

CCND1

It is involved in the regulation of Cyclin D1 gene expression. Cyclin D1 is a cell cycle regulator often mutated, amplified and over-expressed in various types of cancer. After binding of this lncRNA on RNA-binding protein, consequently inhibition of enzymatic activities of the histone acetyltransferases occurs, leading to silencing of cyclin D1 gene. These studies suggest that this lncRNA is a tumour suppressor RNA, which can be rapidly induced by cellular stress to regulate it sense gene expression [85].

6. Conclusion

The rest of ncRNAs, other than miRNAs, in regulation biological functions are more or less unexplored, and this should be further investigated in future research. Regarding therapeutic approaches, we still need more knowledge concerning which miRNAs to target, how to produce and stabilize them, how to direct them to the target tissue. The specificity of drug-like oligonucleotides is important, because of the off-target effect. The off-target effect is also a significant challenge, especially considering that miRNA-mediated repression often requires a homology of only six to seven nucleotides in the seed region of the miRNA and mRNA target site. Toxicity due to chemical modifications, which is used to facilitate cellular uptake and prevent degradation, should be take into account. However, only recently was described the possibility of using exosomes and exosomal tumour-suppressive miRNAs as novel cancer therapy [86].

Author details

Emanuela Boštjančič and Damjan Glavač

Department of Molecular Genetics, Institute of Pathology, Faculty of Medicine Ljubljana, University of Ljubljana, Slovenia

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