Suresh Alahari *Editor*

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Preface

Cancer is a complex and multistep process involving the accumulation of multiple changes that eventually transform normal cells into cancer cells. These changes include structural and expression abnormalities of both coding and non-coding genes. Most cancer-related deaths are not caused by primary tumors but by the spread of cancer cells from the original site to distant sites. In the year 1993, Ambros and colleagues first discovered a gene for lin-4, which did not code for protein, in *C.elegans*, and it was named as microRNAs. Since then several microRNAs have been discovered in various organisms. MicroRNAs have regulatory roles in several biological processes. In cancer, microRNAs function as regulatory molecules acting as oncogenes and tumor suppressors resulting in them having very significant roles in cancer biology. Thus when Springer asked me to work on this book, I accepted the invitation without any second thoughts. Many outstanding investigators have done great amounts of work on microRNA in cancer so we could not cover every study because of space limitations for which we apologize. Our understanding of microRNA's role in cancer is great due to the advent of several genetic engineering approaches through making transgenic and knockout animals for microRNAs. Furthermore, several novel therapeutic modalities for microRNA have reinvigorated many hopes for the cure to cancer. In the last few years microRNA research has grown tremendously, allowing us to get closer to the development of microRNA targeted therapies the usage of microRNAs as diagnostic and prognostic markers. Some microRNAs are detected in the plasma of cancer patients and can serve as diagnostic markers, prognostic markers, therapeutic targets, and causal factors in cancers. The novel microRNA based therapies will likely reduce the incidence of death from cancers. In this book, my goal is to comprehensively review the fundamental knowledge of microRNAs in cancer.

This book is composed of eight chapters that give basic information of the role of microRNAs in cancers. The first chapter describes the general functions of microRNAs and other non-coding RNAs in cancers. Here, authors effectively describe the pivotal role of microRNAs in various malignancies. More importantly, the authors introduce novel non-coding RNAs including MALAT1, HOTAIR and others. The second chapter describes how microRNAs regulate cell proliferation in which authors provide a detailed list of microRNAs that are important in cell

proliferation and discuss, in detail, various therapeutic approaches describing the restoration of tumor suppressor microRNA expression and suppression oncogenic microRNAs expression. In the third chapter, the author elucidates the importance of microRNAs in cancer stem cells. He elegantly narrates the cancer stem cell hypothesis, shows links between cancer stem cells and epithelial-mesenchymal transition, and depicts the important role of microRNAs in normal as well as cancer stem cells. The fourth chapter describes how microRNAs regulate viral pathogenesis and cancers including the methods by which viruses regulate microRNA and viral microRNAs regulate host genes. The fifth chapter deals exclusively with oncogenic microRNAs and describes how they function in normal cells and in cancer cells. It also discusses the cell specific microRNAs and shows the importance of microRNAs in resistance to chemotherapy and radiation therapy. The sixth chapter mainly focuses on metastasis specifc microRNAs. The seventh chapter highlights the role of microRNAs in Leukemias. Finally, the eighth chapter describes various novel approaches for making small molecule modifiers of microRNAs that can be used as molecular probes or in therapeutics and the various methods of the delivery of such small molecules. This chapter is a completely new twist from the current thinking concerning microRNAs.

The authors have done a fantastic job in presenting these complex topics in an easy, understandable manner. I am very thankful to the authors who have written these chapters and unselfishly assisted me in my first editing of a book. I would also like to thank the staff at Springer Science located in the Netherlands, especially Ilse Hensen for her assistance in this process. Finally, I would like to dedicate this book to my father, the late Venkaiah Alahari, and my mother, Saraswathi Alahari, who have supported me in every step of my life with whatever little resources they had and without their help I would not be the individual I am today.

Contents

MicroRNAs and Other Non-Coding RNAs: Implications for Cancer Patients

Reinhold Munker and George A. Calin

Abstract The discovery of microRNAs (miRNAs) has shed new light on the role of RNA in gene regulation. MiRNAs are small molecules (size, 19–22 nucleotides) that do not encode proteins but interfere with translation and transcription, thereby regulating gene expression. Multiple miRNAs are dysregulated in human cancer, supporting the hypothesis that miRNAs are involved in the initiation and progression of cancer. Prototypic malignancies in which a role for miRNAs has been demonstrated include chronic lymphocytic leukemia, multiple myeloma, cutaneous T-cell lymphoma and mantle cell lymphoma. More research is necessary, but miRNAs have already improved our understanding of the pathogenesis of cancer. MiRNAs measured in bodily fluids, especially plasma, may be useful as biomarkers for cancer. Beyond miRNAs, several thousand other non-coding (also called ultraconserved) RNAs may be important in the pathogenesis and prognosis of cancer. Some ultraconserved non-coding RNAs interfere with signal transduction by modifying chromatin structures, but most are not yet well characterized. MiRNAs and other non-coding RNAs may be useful for the gene therapy of cancer.

1 Introduction

The literature on microRNAs (miRNAs), and especially miRNAs in cancer, has increased exponentially over the last 10 years. Cancer is a frequent disease: at least one third of the population will develop cancer during their lifetimes. Despite progress in early detection, chemotherapy, immunotherapy, radiation and other treatments, most people with advanced cancer will ultimately die of the cancer. Overall, new treatments for cancer with fewer side effects are urgently needed. The discovery of miRNAs and other non-coding RNAs will lead to new biomarkers for determining the diagnosis, prognosis, and treatment response of cancer and may ultimately lead to new treatments for cancer.

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It is clear that miRNAs are dysregulated in cancer. For many types of cancer, miRNA signatures have been established. Some signatures provide prognostic information. The field of miRNAs in cancer was launched when Calin et al. [1, 4] showed that miR-15 and miR-16 were located in a region (chromosome 13q14) frequently deleted in chronic lymphocytic leukemia (CLL). Consequently, the expression of miR-15 and miR-16 in CLL is decreased. Subsequently, based on 218 samples, Lu et al. [2] showed that cancer can be classified according to miRNA expression. Based on a larger collection of samples and using a customized microarray, Volinia et al. [3] published a miRNA signature of solid tumors. In this chapter, we give an update on the role of miRNAs in cancer exemplified by important disease entities (CLL, multiple myeloma, cutaneous T-cell lymphoma and mantle cell lymphoma) and then look further into other recent developments in the field of non-coding RNA. We recently published a general overview of the topic of miRNAs in cancer [5].

Fundamentally, miRNAs are small molecules (approximate size, 19–22 nucleotides) that do not encode proteins. The major function of miRNAs is to regulate gene expression. It has been estimated that 30 % or more of mammalian genes are regulated by miRNAs. Mechanisms by which this regulation occurs involve degradation of messenger RNA (mRNA), chromatin-based silencing and inhibition of translation. MiRNAs are highly conserved between different species. Currently, more than 600 miRNAs are known or generally accepted. About half of all known miRNAs are located in minimal regions of amplification, at common breakpoints associated with cancer or in close proximity to fragile sites or in minimal regions of loss of heterozygosity [5].

The synthesis of miRNAs begins in the nucleus at the stage of pri-miRNA transcripts. Subsequently, these transcripts are cleaved by an RNase III-type nuclease (Drosha) and form hairpin structures of 60–70 nucleotides (pre-miRNAs). Pre-miRNAs are exported into the cytoplasm by exportin. In the cytoplasm, the enzyme Dicer performs further cleavage, which results in an asymmetric intermediate (MiRNA: MiRNA*). The duplex then makes contact with the RNA-induced silencing complex (RISC), where one strand becomes active and functional (repressing translation and degrading mRNA). The inactive strand (marked by an asterisk or star) is generally not considered of functional importance (although there may be exceptions [6]). For a detailed review about the biogenesis of miRNAs, see Krol et al. [7].

2 MiRNAs in Selected Malignancies

Among the myriad studies and publications about the significance of miRNAs in cancer, we will discuss here four diseases that are relevant to our current research. CLL is the most frequent leukemia in Western countries and has become the paradigmatic disease for the involvement of miRNAs in cancer. Multiple myeloma is the second most frequent hematologic malignancy; it involves bones and bone

marrow. Cutaneous T-cell lymphoma and mantle cell lymphoma are rare types of T- and B-cell lymphomas with a wide spectrum of clinical presentations and outcomes. In all these diseases and disorders, miRNAs were shown to be important.

2.1 Chronic Lymphocytic Leukemia

A frequent chromosomal aberration in CLL is the homozygous or heterozygous deletion of the chromosomal region 13q14.3. Patients with this deletion often have an indolent or benign clinical course. In 2002, it was shown by Calin et al. [8] that two genes encoding miRNAs (miR-15a and miR-16-1) are located in this region, providing evidence that miRNAs could be involved in the pathogenesis of human cancer [8]. MiR-15a and miR-16-1 map to a 30 kb region between exons 2 and 5 of the DLEU2 gene (which is deleted in these patients). A common hypothesis is that the loss of both miRNAs is an early event in the pathogenesis of CLL.

In a later study, a unique miRNA signature for CLL was defined [9]. The signature of nine miRNAs (eight whose expression was increased, one whose expression was decreased) correlated with somewhat more aggressive disease. This pattern also corresponded to known biologic risk factors for CLL, such as high expression of 70 kDa zeta-associated protein (ZAP70) and unmutated immunoglobulin heavy chain genes.

The role of miRNAs in the predisposition to or inheritance of cancer is another area of research. In support of such a role, mutations of some miRNA genes were found in 11 of 75 patients with CLL. This discovery points to a genetic disposition for cancer in some patients with CLL.

The New Zealand Black mouse model of CLL supports the role of miRNAs in the pathogenesis of CLL. In this model, a 3' point mutation adjacent to miR-16-1 led to reduced expression of miR-16-1 [10]. In a different mouse model, the deletion of the 13q14 minimal deleted region (encoding the DLEU2/miR-15a/16-1 cluster) caused development of indolent B-cell–autonomous and other clonal lymphoproliferative disorders. This deletion recapitulates the spectrum of CLL-associated phenotypes observed in patients [11]. The loss of miR-15a/16-1 accelerates the proliferation of B lymphocytes both in mice and humans by modulating the expression of genes controlling cell-cycle progression. A mouse model for indolent CLL was recently generated by overexpressing miR-29 in B cells. Such Eμ-miR-29 transgenic mice developed CD5 + B lymphocytosis starting at 2 months of age. By 2 years, the percentage of CD5 + B lymphocytes had increased to 100 %, and about 20 % of the mice died from leukemia [12].

Patients with cancer or leukemia often respond to chemotherapy, but later relapse and become resistant. The topic of resistance to cancer chemotherapy is clinically relevant and may involve miRNAs. The phenotype of *in vivo* fludarabine resistance was described as upregulation of miR-18, miR-122 and miR-21 [13]. The authors studied 723 miRNAs in 17 patients with CLL. RNA was harvested from peripheral blood before and after a 5 day course of fludarabine. Nine patients responded

clinically, eight patients were classified as resistant. In responding patients, the activation of p53 responsive genes was detected.

Feedback circuitry linking miRNAs, TP53 and the pathogenesis and outcome of CLL was established by Fabbri et al. [14]. For this study, CLL Research Consortium institutions provided 206 blood samples from untreated patients with B-cell CLL. These samples were evaluated for the occurrence of cytogenetic abnormalities, as well as the expression levels of the miR-15a/16-1 cluster, miR-34b/34c cluster, TP53 and ZAP70. The functional relationship between these genes was studied using *in vitro* experiments examining gain and loss of function and was validated in a separate collection of primary CLL samples. In 13q-deleted samples (as mentioned, associated with a favorable prognosis), the miR-15a/16-1 cluster directly targeted TP53 and its downstream effectors. In leukemic cell lines and primary B-CLL cells, TP53 stimulated the transcription of both miR-15/16–1 and miR-34b/34c clusters, and the miR-34b/34c cluster directly targeted ZAP70 kinase.

The interplay between protein-coding genes and miRNAs, as well as other non-coding RNAs, in CLL was reviewed by Calin and Croce [15].

2.2 Multiple Myeloma

The first study involving miRNAs in multiple myeloma showed that interleukin-6 induces miR-21 via Stat3 activation. When miR-21 was increased ectopically, the myeloma cells lost their interleukin-6 dependence [16]. Pichiorri et al. [17] in 2008 were first to establish an miRNA expression profile for multiple myeloma by comparing myeloma cell lines with CD138-selected samples from patients with myeloma, samples from patients with monoclonal gammopathy of unknown significance, and normal plasma cells. In these profiles, miR-21, the miR-106b~25 cluster and miR-181a/b measured in patients' bone marrow myeloma cells were overexpressed compared with expression in normal plasma cells. Two miRNAs, miR-19a/b, which are part of the miR17~92 cluster, were shown to interact with the expression of the SOCS-1 gene. In addition, xenograft studies implicated miR-19a/b and miR-181a/b in the pathogenesis of multiple myeloma [17]. This work was recently extended by demonstrating that miR-192, miR-194 and miR-215 (which are often downregulated in newly diagnosed multiple myeloma) are part of an autoregulatory loop with MDM2 and p53. It was shown that through small-molecule inhibitors of MDM2, these miRNAs can be transcriptionally activated by p53 and then modulate MDM2. In addition, miR-192 and miR-215 target the IGF pathway, preventing the homing of myeloma cells [18]. The correlation between miRNA expression, DNA copy number changes and gene expression was studied by Lionetti et al. [19]. A new histone deacetylase inhibitor (ITF2355) was shown to downregulate miR-19a and miR-19b [20]. In 15 patients with relapsed or refractory myeloma, a decrease of miR-15a and miR-16 and an increase of miR-222, miR-221 and miR-382 were found [21]. In a larger study involving 52 newly diagnosed patients, a global increase in miRNA expression was observed in high-risk disease. High-risk disease was defined by a

70-gene risk score and the proliferation index. Of particular interest is that one of these genes, EIF2C2/AGO2, is considered to be a master regulator of the maturation and function of miRNAs. When EIF2C2/AGO2 was silenced, the viability of multiple myeloma cell lines decreased dramatically [22].

2.3 Cutaneous T-Cell Lymphoma

Recently, a miRNA expression profile for Sézary syndrome, the leukemic form of cutaneous T-cell lymphoma, was established [23]. Sézary syndrome generally has a poor prognosis. Most miRNAs expressed in Sézary syndrome were downregulated and distinguished Sézary syndrome both from normal CD4-positive T cells and from B-cell lymphomas. The authors showed that downregulated miR-342 inhibits apoptosis, thereby suggesting a role for this miRNA in the pathogenesis of cutaneous T-cell lymphoma. The work on cutaneous T-cell lymphoma needs to be extended. Of special interest will be whether an *in vivo* resistance profile to histone deacetylase inhibitors can be determined.

2.4 Mantle Cell Lymphoma

Mantle cell lymphoma has a well-defined chromosomal marker, t(11; 14), which leads to overexpression of cyclin D1. Zhao et al. [24] performed expression profiling for 30 patients with mantle cell lymphoma and found a decrease in 18 miRNAs and an increase in 21 miRNAs compared with levels in normal B lymphocytes. The authors demonstrated that miR-29 inhibits CDK6 protein and mRNA (which are involved in the pathogenesis of mantle cell lymphoma) by binding directly to the 3'-untranslated region of the mRNA. In addition, they showed that cases with the lowest miR-29 levels in lymphoma cells had the worst prognosis [24]. In a different profiling study of miRNA expression in mantle cell lymphoma (involving eight cell lines and a total of 77 patients), increases in miR-106b, miR-93 and miR-25 were demonstrated (among other changes) [25].

3 Soluble MiRNAs

Plasma or serum tumor markers could enable detection of cancer without invasive procedures. Mitchell and colleagues [26] reported that plasma contains stable miRNAs that are protected from endogenous RNase activity. The use of miRNAs as tumor markers was exemplified by their measurements of miR-141 derived from prostate xenografts. The authors also demonstrated that levels of this miRNA could be used to distinguish patients with prostate cancer from healthy controls. In

colorectal cancer, one study showed that levels of two plasma miRNAs were significantly increased in comparison to levels in normal individuals (and decreased after surgery). A possible use of such plasma-based miRNAs might be screening for colorectal carcinoma [27]. Plasma miRNAs were also applied to non–small cell lung cancer [28]. The miRNAs miR-200a/b are often overexpressed in biopsies of pancreatic cancer. Li et al. [29] recently investigated serum miRNAs in pancreatic cancer and found that levels of miR-200a/b in most patients were elevated compared with levels in normal controls. The diagnostic value of miR-200a/b is doubtful, however, because a similar elevation was observed in chronic pancreatitis [29]. In a further study, levels of three miRNAs were increased in whole blood from cancer patients; and one of these miRNAs (miR-195) appeared specific for breast cancer [30]. When 12 different types of bodily fluids (from plasma to colostrum) were investigated, miRNAs were found to be ubiquitous. It was speculated that some miRNAs may transmit signals between cells and tissues [31]. Taken together, the studies on soluble miRNAs have mostly been done with small patient numbers and controls and would need standardization before clinical use could be considered. Nevertheless, if the data are reproducible and valid when all controls are integrated in the protocol, plasma miRNAs may be a simple way of diagnosing cancer.

4 Other Non-coding RNAs

Up to 70 % of the human genome is transcribed, but only 2 % of the genes are translated into proteins. Besides miRNAs, which we have begun to understand, there are many other non-coding RNAs (probably more than 6,000), most of which are not well characterized. Some of these molecules may be important in the regulation of gene expression and, by proxy, also in the pathogenesis and progression of cancer. These molecules may also serve as new biomarkers.

We will discuss here the other non-coding RNAs for which an involvement in cancer has been shown. These include long intergenic non-coding RNAs (lincRNAs), such as MALAT-1, HOTAIR and other transcribed non-coding RNAs. By definition lincRNAs are molecules of more than 200 nucleotides in length. One category of lincRNAS, the ultraconserved non-coding RNAs are identical between mouse, rat and man and therefore deemed important in gene regulation. The mechanisms of gene silencing by lincRNAs may involve epigenetic modifications of chromatin within promoter regions [32]. It was recently reported that a large fraction of genomic ultraconserved regions encode a particular set of non-coding RNAs whose expression (similar to miRNAs) is altered in human leukemias and cancers. Ultraconserved regions are frequently located at fragile sites and genomic regions involved in cancer. These non-coding RNAs may be regulated by miRNAs that are abnormally expressed in human CLL [33]. Similar to miRNAs, the non-coding RNAs transcribed from ultraconserved regions are often hypermethylated in human cancer [34]. According to Huarte and Rinn [35], lincRNAs may provide the "miss-

ing link in cancer," implying that these molecules could also function as tumor suppressors and tumor inducers and thereby initiate or promote cancer.

4.1 MALAT-1

MALAT-1 was originally isolated by subtractive hybridization from a pool of metastatic lung adenocarcinomas [36]. The MALAT-1 transcript has 8,000 nucleotides, originates from human chromosome 11q13 and is conserved across several species. MALAT-1 is expressed in several normal tissues, such as the pancreas and lung, and overexpressed in metastatic lung cancer. The overexpression of MALAT-1 in early lung cancer predicts ultimate metastasis and death from metastatic lung cancer. The mouse ortholog of MALAT-1, designated as hepcarcin, was found to be strongly expressed in mouse carcinogen-induced liver cancers, as well as human hepatocellular carcinomas [37]. In osteosarcoma, high expression of MALAT-1 corresponded with poor response to chemotherapy [38]. Recently, it was shown that the silencing of MALAT-1 impaired the motility of lung cancer cells, which may explain the role of MALAT-1 in metastasis. The knockdown of MALAT-1 influenced the expression of numerous genes (including CTHRC1, CCT4, HMMR and ROD1, which on their own also influence cell motility) [39].

4.2 HOTAIR

The lincRNA HOTAIR is in the mammalian HOXC locus and binds to and targets the PRC2 complex on the HOXD locus, which is located on a different chromosome. It was recently shown that HOTAIR is overexpressed a hundred—to a thousand-fold in breast cancer metastases. In primary tumors, HOTAIR expression is a powerful predictor of eventual metastasis and death. The enforced expression of HOTAIR leads to a genome-wide re-targeting of PRC2 to an occupancy pattern resembling embryonic fibroblasts and increased cancer invasiveness and metastasis. These findings suggest that lincRNAs have active roles in modulating the cancer epigenome and may become important targets for the diagnosis and treatment of cancer [40].

4.3 H19

The H19 locus is subject to genomic imprinting and produces a 2.5 kb non-coding, spliced and polyadenylated RNA. It was recently shown that this locus acts as an *in vivo* tumor suppressor in several mouse models of cancer [41].

4.4 *XIST*

XIST is a non-coding transcript involved in X chromosome silencing. Some recent data point toward an involvement in breast cancer, especially in BCRA-1-related cases [42].

4.5 *SnaR Family Members*

Members of the snaR family of small non-coding RNAs associate *in vivo* with nuclear factor 90 protein. The major human species (snaR-A) has a restricted tissue distribution (brain, testis and some other tissues) and is upregulated in transformed and immortalized cells. In the HeLa cell line, snaR-A is stably bound to ribosomes [43].

4.6 *Other Transcribed Ultraconserved Regions*

Transcribed ultraconserved regions were shown to be widely expressed in neuroblastomas, and their expression correlates with important clinicogenetic parameters such as N-MYC amplification [44]. Recently, a novel lincRNA in 8q24 was described. This lincRNA has a size of approximately 13 kb. The authors found several single nucleotide polymorphisms that increased the risk for prostate cancer in Japanese patients; they termed this novel RNA "prostate cancer non-coding RNA1". Knockdown of this novel RNA by siRNA decreased both the viability of prostate cancer cells and the transactivation of the androgen receptor [45]. The HULC gene ("highly upregulated in liver cancer") is a non-coding RNA transcribed from human chromosome 6p24.3. HULC is strongly expressed in hepatocellular carcinomas but also to a lesser extent in normal hepatocytes. It was recently shown that HULC is also expressed in liver metastasis of colon cancer but not in primary colon cancers [46]. The PCGEM1 non-coding gene (previously described as prostate-specific and androgen-regulated) was recently shown to play a role in the *in vivo* progression of prostate cancer [47]. In liver cancer, a transcribed non-coding RNA was shown to modulate cell growth [48]. This lincRNA (TUC338) is predominantly located in the nucleus and has strongly increased expression in carcinoma cells compared to non-transformed hepatocytes. TUC338 is located in part within another gene (poly (rC) binding protein 2), but transcribed independently.

5 Implications for the Treatment of Cancer

Ultimately, the study of miRNAs and other non-coding RNAs can only make an impact on clinical hematology and oncology if new and better treatments for cancer can be developed. This goal can also be achieved by prognosticating new risk

factors and better targeting the currently available treatments for cancer. Possibilities are to introduce tumor suppressor non-coding RNAs directly into cancer cells or to antagonize over-expressed cancer-promoting non-coding RNAs in cancer cells. Worthwhile topics of study are how resistance mechanisms can be overcome and how the currently available treatments for cancer (radiation, chemotherapy, cytokines, small molecules) interact with the non-coding RNAs in cancer cells. A potential advantage of gene therapy using miRNAs or similar non-coding molecules is that these molecules can be easily transfected because of their size. In addition, because one non-coding molecule regulates multiple genes, small changes in that molecule's expression *in vivo* may have a major impact on cancer signal transduction. Potential disadvantages of gene therapy may be lack of specificity and stability of the transfected molecules. Potential side effects also need careful attention.

The first step in testing a new treatment in humans is to study the drug or procedure in animal models, for example, in immunosuppressed (nude) mice. As an example, the transfection of myeloma cell lines with miR-19 and miR-181 antagonists resulted in significant tumor suppression in a xenograft mouse model [17]. In glioma, the transfection of the precursor of miR-34 into a glioma cell line led to a drastic reduction of tumor growth when injected into the brain of immunosuppressed mice [49].

Another miRNA that has been tested preclinically is miR-34a, which has reduced expression in several types of cancer, including lung cancer, and is considered a tumor suppressor miRNA. A group of scientists recently synthesized a miR-34a mimic and incorporated it into a lipid-based vector. When miR-34a was administered into tumors or into the systemic circulation in mice, the development of lung tumors was delayed or blocked. The authors showed that miR-34 accumulated in the tumors and its direct targets were downregulated. In this mouse model, few side effects were observed; in particular, no elevations of liver enzymes or cytokines occurred [50].

MiR-191 was recently identified as a potential target for gene therapy in hepatocellular carcinoma. In vitro, the inhibition of miR-191 decreased cell proliferation and induced apoptosis. In vivo, in an orthotopic xenograft mouse model, anti-miR-191 significantly reduced tumor masses. In addition, miR-191 was found to be upregulated by a known liver carcinogen (dioxin) and regulated various cancer-related pathways [51].

Especially in aggressive cancers, chemotherapy or radiation eradicates more than 98 % of tumor cells, but due to cancer stem cells, the cancer re-grows, develops metastasis and leads to death. Targeting miRNAs or other non-coding RNAs to cancer stem cells, alone or in combination with currently available treatments for cancer, would constitute a breakthrough in cancer therapy.

In disorders other than cancer (hepatitis, hypercholesterolemia), interesting preclinical models have been published. The miRNA miR-122 is expressed predominantly in liver cells and is essential for hepatitis C RNA replication. Chimpanzees chronically infected with hepatitis C were treated with modified oligonucleotides complementary to miR-122. This treatment led to a long-lasting decrease in the hepatitis C viral load (without increased resistance), accompanied by suppression of miR-122 [52]. At the same time, interferon-regulated genes were modulated.

In summary, the safe and effective administration of miRNAs and antagomirs in patients with cancer would have a major impact. Before this can happen, more work elucidating pathomechanisms and optimizing delivery of miRNAs and other non-coding RNAs is necessary. From the point of view of drug development, frequent cancers (such as lung cancer or breast cancer) and cancers for which no effective treatment is available for advanced stages (such as malignant melanoma or hepatocellular carcinoma) will have priority. Combining our growing understanding of non-coding RNA with the data from whole-genome sequencing, a clearer perspective of what causes cancer is on the horizon.

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