Short Conceptual Overview

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Suggested roles for microRNA in tumors

Abstract: MicroRNAs are short non-coding RNA molecules encoded by distinct genes involved in the posttranscriptional regulation of gene expression. Forming part of the epigenetic machinery, microRNAs are involved in several aspects of tumorigenesis. Deregulation of microRNA expression is a common feature of tumors. Overexpressed oncogenic and underexpressed tumor suppressor microRNAs have been described in many different tumors. MicroRNAs are released from tumors that might affect other cells within and outside the tumor. Circulating microRNAs might also be involved in a tumor surveillance mechanism. In this short overview, some important aspects of microRNA in tumors are discussed.

Keywords: circulating; epigenetic; membrane vesicle; microRNA; oncogene; tumor; tumor suppressor.

Introduction

MicroRNAs (miR, miRNA) are short non-coding ribonucleic acid molecules primarily involved in the postranscriptional regulation of gene expression (1). MicroRNAs were discovered as endogenous mediators of RNA interference: mature single-stranded microRNA bind to the 3′ untranslated region (3′ UTR) of messenger RNA (mRNA) molecules and thereby inhibit their translation or induce their degradation (1). MicroRNA are encoded by distinct genes that are mostly located in non-coding parts in the genome (the former 'dark matter'), and also many are found in introns

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of protein coding genes (2). MicroRNA genes are often found in clusters that often comprise microRNAs of similar sequence or function (microRNA families) (3). Because microRNAs affect gene expression without altering the nucleotide sequence, they can be considered parts of the epigenetic machinery. The number of microRNAs is steadily increasing, and their number exceeds 2500 in humans at present (2588 mature microRNAs in the miRBase registry: www.mirbase.org, release June 21, 2014).

A sophisticated maturation process gives rise to mature microRNAs, whereby primary hairpin structure transcripts of microRNA genes synthesized by RNA polymerase II, called pri-microRNA are first cleaved by intranuclear RNAse III Drosha in complex with DGCR8, and then the resulting pre-microRNAs are transported into the cytoplasm via the nuclear membrane by Exportin 5/ Ran GTPase (XPO5) proteins. Mature 19–25 nucleotide long microRNAs are produced from pre-microRNAs by cytoplasmic Dicer. Mature single stranded microRNAs are stabilized in the RISC (RNA-induced silencing complex) containing transactivation-responsive RNA-binding protein (TRBP) and Argonaute 2 (Ago2) as major components and several other factors associated to Ago proteins such as helicases (4, 5) (Figure 1).

MicroRNAs are very well-conserved molecules that can be found in a wide range of species ranging from viruses and plants to mammals. MicroRNAs mostly negatively regulate gene expression by inhibiting mRNA translation or inducing their degradation; however, there are data that in some cases show that microRNA might even promote translation (6). A single microRNA can target several hundreds of different mRNAs, thus the action of microRNA is pleiotropic (4). Moreover, a single mRNA can be targeted by several different microRNAs, whose action is often synergistic (1). This complex interaction between microRNAs and target mRNAs constitutes very complicated interaction networks that appear to be tissue specific. MicroRNAs are predicted to regulate 30%–60% of human protein coding genes (7–9).

Apart from this classic mRNA-targeting action of microRNA taking place in the cytoplasm, novel data show that microRNA have other basic functions as well. MicroRNAs have been shown to affect gene transcription
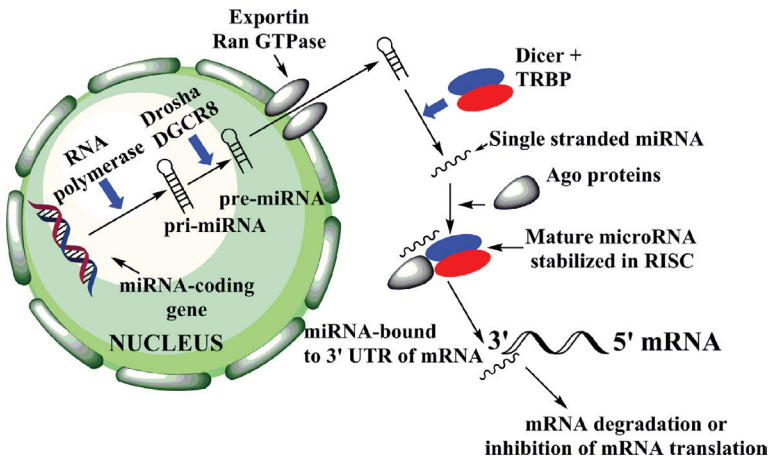
Figure 1: Biogenesis and maturation of microRNA.

directly in the nucleus (10) and thereby participate in forming the genomic landscape of transcription (7). MicroRNAs also influence other epigenetic pathways including chromatin remodeling, methylation, etc. (7). Conversely, the expression of microRNAs is also affected by epigenetic pathways (7).

MicroRNAs affect several basic biological phenomena via targeting crucial mRNAs involved in the regulation of cell proliferation, differentiation, apoptosis, ontogenesis, immune regulation, etc. (11). Given their basic biological functions, it was not surprising to uncover their relevance in several diseases. The differential expression of microRNA has been described in a wide variety of diseases including cancer (3, 12). MicroRNA deregulation is considered to be a major phenomenon of tumor formation (3, 4). Significant differences of microRNA expression have been described between benign and malignant tumors; moreover, subclassification of cancer might be also feasible by microRNA profiles (13). MicroRNA profiling in tumor metastases might be helpful for identifying the primary tumor (14); moreover, microRNA expression changes were identified in the stroma surrounding the tumor that might also be exploited for diagnostic purposes (15). Differential expression of tissue microRNAs can be used for the diagnosis of malignancy or as prognostic markers. MicroRNA markers of malignancy can be especially useful for the diagnosis of tumors whose histological analysis is difficult [e.g., differentiated thyroid tumors (16, 17) and adrenal tumors (12)]. The diagnostic utility of microRNAs is further extended due to their stability, because formalin-fixed, paraffin embedded tissue blocks might also be efficiently used for microRNA analysis (18). In this brief overview, the most important aspects of microRNA in tumors are discussed.

MicroRNA as oncogenes and tumor suppressors

MicroRNA deregulation is considered to be an early event in tumorigenesis (19). Altered expression of microRNAs was described even in the histologically normal tissue surrounding the tumor, e.g., in papillary thyroid carcinoma (16). Several microRNAs in tumors have been found to be over- or underexpressed relative to normal tissues. Following the classical tumor suppressor-oncogene dichotomy, underexpressed microRNAs are termed tumor suppressors, whereas overexpressed are considered to be oncogenic (oncomiR) (20). However, as most cytoplasmic actions of microRNAs are exerted via targeting various messenger RNAs, overexpressed oncogenic microRNAs actually mostly target tumor suppressor mRNAs, whereas the underexpression of tumor suppressor microRNAs results in the overexpression of oncogenic mRNAs (20) (Figure 2).

About 50% of microRNA genes are located in fragile chromosomal regions that are often damaged in tumor tissues (21). However, the association with copy number variations and microRNA expression alterations is not clear cut (22).

In the first study describing the loss of a tumor suppressor microRNA gene involved in disease pathogenesis, *miR-15* and *miR-16* expression was found to be down-regulated in chronic lymphocytic leukemia (23). Both *miR-15* and *miR-16* were revealed to target the anti-apoptotic gene *BCL-2* (B-cell leukemia 2), and therefore their underexpression resulted in *BCL-2* overexpression leading to increased cell proliferation (24, 25). Later, several other microRNA genes have been found with predominant
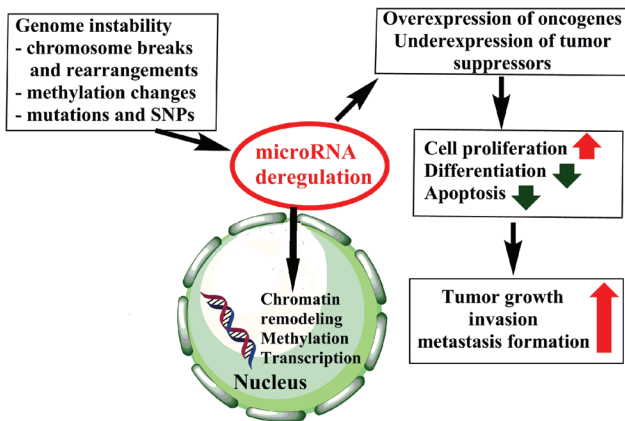
Figure 2: Schematic representation of the relevance of microRNA dysregulation in tumors.
Green arrows represent inhibition, red arrows stimulation.

tumor suppressor or oncogenic activity such as the class of *let-7* microRNAs that are mainly tumor suppressors (26) or *miR-21* that is mainly oncogenic (27).

The oncogene-tumor-suppressor dichotomy is, however, further complicated by the tissue specific action of microRNAs. The same microRNA can be tumor suppressor in one tissue but oncogenic in another. For example, *miR-503* is overexpressed in adrenocortical cancer (28) but underexpressed in pituitary tumors (29). There are numerous examples of such discrepancies among various tumors (30). This tissue specific behavior of microRNAs may represent a major hurdle in the development of microRNA-based treatment for cancer.

The expression of microRNA is influenced by transcription factors that, in turn, can be targeted by microRNAs. Interesting regulatory loops may thus arise, such as the regulatory network including the *c-MYC* protooncogene, *miR-17-5p* and *miR-20a* and the transcription factor *E2F1* (31). In this network, *c-MYC* induces the transcription of both *E2F1* and the *miR17-92* cluster including these microRNAs, but *miR-17-5p* and *miR-20a* target the E2F1 mRNA, whereby a fine control of *E2F1* activity might be achieved (31). The *TP53* (tumor protein 53) is another major transcription factor that is often lost in cancer tissues. Because *TP53* induces the transcription of *miR-34a* that is mostly a tumor suppressor microRNA inducing growth arrest in several cell lines and tissues, loss of *TP53* might be associated with decreased *miR-34a* and thus increased cell proliferation (32). Moreover, *TP53* appears to modulate microRNA processing and this phenomenon was suggested to take part in its tumor suppressive activity (33). Conversely, the expression of *miR-34a* was shown to be affected by a *TP53*-independent pathway too, as the

ETS family transcription factor, *ELK1*, was found to induce *miR-34a* expression during oncogene induced senescence (34); *c-MYC* is known to be among the targets for *miR-34a* (34). These examples highlight the complex interactions among different players of the gene expression regulation machinery including both transcription factors and microRNAs as posttranscriptional mediators. Fine-tuned regulation of cell cycle and other basic cellular phenomena is based on these sophisticated interactions. Derailment of these balanced regulatory networks can be observed in malignant transformation.

MicroRNA genes and microRNA processing enzymes affected by mutations and single nucleotide polymorphisms (SNP)

Because the classic mRNA-targeting action of microRNAs involves base pairing between the microRNA and the target mRNA, genetic variation in either the microRNA or its recognition sequence might affect the activity of microRNAs (35). The first example of this phenomenon was again delivered by chronic lymphocytic leukemia, where a mutation in the pri-microRNA sequence of *miR-16* was found in a familial case of the disease (36). This was an interesting example of an inherited germ-line mutation affecting a non-coding RNA involved in gene expression regulation. In contrast with mutations, single nucleotide polymorphisms (SNP) are much more frequent (found at approximately every 1000 nucleotides of the genome). Variation in both microRNA and target sequences appears to be involved in a wide variety of human cancers (35) and might affect drug sensitivity and treatment response as well. Moreover, there are data that the biogenesis of microRNA might be affected by SNPs in microRNA processing enzymes, and these could be associated with some tumors (35). Thus, the SNP profile of an individual might affect the microRNA expression profile (miRNome). There are several data on the potential association of certain microRNA sequence or target sequence SNPs and tumor risk (35).

Considering mutations of microRNA processing enzymes, frequent hemizygous deletions of Dicer1 resulting in haploinsufficiency has been described in human cancers (37, 38), and Dicer1 can thus be regarded as a tumor suppressor (38). Somatic mutations including a recurrent mutation of Drosha and other microRNA processing enzyme genes have been noted in Wilms tumor samples (39).

It can be hypothesized that due to the genomic instability characteristic for malignant tumors (40), the accumulation of genetic variants and mutations might affect the biogenesis and action of microRNAs that could be relevant in tumor progression. Moreover, the microRNA target mRNAs are also more prone to alternative splicing in proliferating cells that might result in shorter 3′ UTR sequences harboring fewer microRNA binding sites (41).

MicroRNA released from tumor cells

Apart from tissue microRNAs, novel data show that microRNAs are released from cells and enter body fluids and excrements (blood, urine, feces, saliva, semen, milk, etc.) (42). MicroRNAs are released in three major ways: (i) passive release through damage (necrosis, inflammation), (ii) active release packed in extracellular membrane vesicles (microvesicles, exosomes, and apoptotic bodies), or (iii) associated with protein complexes such as Argonaute 2 and high density lipoprotein (HDL) (43). The microRNAs released in the blood are surprisingly stable and the circulating microRNAs can be exploited as minimally invasive biomarkers of tumors even in their very early stages (44). Blood-borne circulating microRNA might be regarded as hormones conveying epigenetic information to distant tissues (19) (Figure 3). Circulating microRNA markers are very promising biomarkers that could be used as minimally invasive biomarkers of tumor malignancy or prognosis (44).
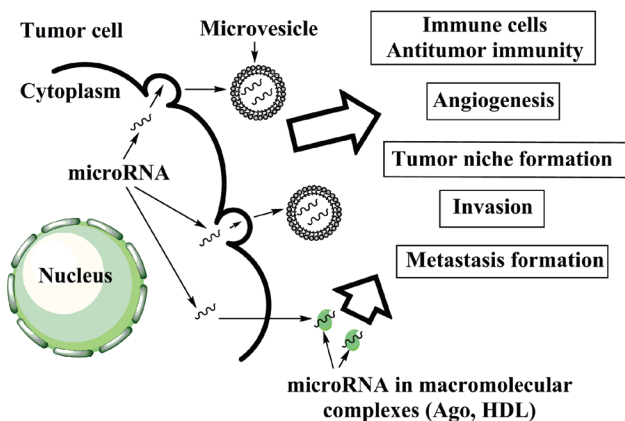
Figure 3: Relevance for tumor-secreted microRNAs in tumor pathogenesis and progression.
MicroRNA in extracellular vesicles might enter other tumor and normal cells. MicroRNA in macromolecular complexes might also be relevant, but these warrant experimental validation, as well.

A growing body of experimental evidence shows that tumor cells release microRNAs in extracellular membrane vesicles, and the activity of this release process seems to be correlated with tumor stage, i.e., advanced tumors release more extracellular vesicles. These extracellular vesicles are very important in the communication among tumor cells but also with the surrounding normal tissue and immune cells (45). In a rat model, exosome-derived microRNAs were shown to affect non-transformed cells by modulating the expression of proteases, adhesion molecules, etc., and so might be involved in tumor invasion and metastasis formation (46); *miR-1227* contained in large extracellular vesicles (oncosomes) released from a prostate cancer cell line enhanced the migration of cancer associated fibroblasts (47). Beside microRNAs, extracellular vesicles contain mRNAs and proteins as well. The vesicles might fuse with cell membranes of other cells, whereby their contents might enter these cells. The entry of microRNA into recipient cells could be involved in the epigenetic reprogramming of these cells (45, 48, 49).

Circulating microRNA constituting a tumor surveillance mechanism?

By looking through the relative abundance of circulating microRNAs in the blood of healthy individuals, we have noted a relative abundance of microRNAs with predominant tumor suppressor activity, e.g., *miR-451*, *miR-223*, *let-7*, *miR-16* (50). Based on this observation, we have raised a novel hypothesis claiming that the relative abundance of tumor suppressor microRNAs in the circulation might represent a tumor surveillance mechanism. There are experimental data that tumor-cell-derived extracellular vesicles might enter other cells (both tumor and normal) (45), but the activity of microRNAs in recipient cells warrants experimental validation. We hypothesize that circulating tumor suppressor microRNAs packed in membrane vesicles (or perhaps in macromolecular complexes, but this awaits experimental validation) entering transforming cells might halt their transformation in their early phase or induce their apoptosis. Such a circulating microRNA-mediated tumor surveillance might complement the well-known immune tumor surveillance or cancer immunoediting (51). There are, however, microRNAs with predominant oncogenic activity in the circulation, such as *miR-21*, and the tissue specific activity of microRNAs can be raised also as a counter arguments against this hypothesis. It is not clear at present how efficient these circulating microRNAs might be as tumor repressors, and

intensive experimental work-up is needed to confirm this hypothesis (50).

The relative abundance of predominantly tumor suppressive circulating microRNAs has been observed in advanced cancer patients, too. In a hypothesis put forward by Chen et al., the abundance of tumor suppressor microRNAs was suggested to represent an anti-cancer activity defense mechanism (52). As most deregulated microRNAs were shown to be implicated in the regulation of the immune response, this anti-cancer activity might affect the immune system.

MicroRNA in anti-tumor therapy

Given their major cell biological roles and relevance in tumorigenesis, intensive efforts are underway to target microRNAs for treatment purposes. Based on the onco-gene-tumor suppressor dichotomy, two major directions can be taken: (i) to decrease the activity of oncogenic microRNAs, and (ii) to enhance or replace the missing activity of tumor suppressor microRNAs. Given the often synergistic action of microRNAs, it could be useful to target several microRNAs simultaneously.

There are some promising *in vitro* and animal data on the anti-tumor application of microRNAs, e.g., over-expressed *miR-21*, *miR-221*, and *miR-214* (53), but intro-duction to human clinical treatment is still far away. In adrenocortical tumors, we have proposed that *miR-483-5p/miR-483-3p*, *miR-195*, and *miR-210* would be the most suit-able candidates for treatment targets (12). Modulation of microRNAs might facilitate other anti-tumor treatments, e.g., the up-regulation of *miR-195* in breast cancer cells enhanced their sensitivity to adriamycin administration (54), or down-regulation of the main hypoxia-associated *miR-210* improved the outcome of radiation therapy in a human hepatoma xenograft model (55).

There are several available molecular techniques that could make these goals feasible in the clinical setting, e.g., microRNA mimics for replacing tumor suppressors or antagomiRs to counteract overexpressed oncogenic microRNAs (56). There are, however, major problems to overcome. Technical issues include the efficient adminis-tration of microRNA (liposomes, plasmids, viral vectors, nanoparticles) and the dose to be used. A major biological difficulty is related to the tissue specific action of micro-RNA, whereby off-target actions may arise in other organs or tissues with undesirable side effects. If the dose of the administered pre-microRNA is too high, it might saturate the endogenous microRNA processing machinery that might lead to unforeseen complications (12, 56).

Conclusions

Since their discovery two decades ago, microRNAs have experienced an unprecedented career in molecular medi-cine including oncology. Altered expression of microRNA appears to be a cornerstone of tumor formation and can be exploited in tumor diagnosis and even in therapy. It must be emphasized, however, that microRNAs act together with other molecular players, and the different layers of molecular alterations including the genome, miRNome, transcription factors, proteome, methylome, and metabo-lome interact with each other. Major developments are to be expected in the field of microRNAs in tumor biology, including their roles in pathogenesis, diagnosis, and as treatment targets. The advent of next generation sequenc-ing and novel bioinformatics approaches might help to unravel this fascinating field of biology.

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