Acta Biochim Biophys Sin 2011, 43: 831–839 | © The Author 2011. Published by ABBS Editorial Office in association with Oxford University Press on behalf of the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. DOI: 10.1093/abbs/gmr080.
Advance Access Publication 10 September 2011

Review

New role of microRNA: carcinogenesis and clinical application in cancer

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MicroRNA (miRNA) is a cluster of small non-encoding RNA molecules of 21–23 nucleotides in length, which controls the expression of target gene at the post-transcriptional level. Recent researches have indicated that miRNA plays an essential role in carcinogenesis, such as affecting the cell growth, differentiation, apoptosis, and cell cycle. Nowadays, multiple promising roles of miRNA involved in carcinogenesis are emerging, and it is shown that miRNA closely relates to the process of epithelial–mesenchymal transition (EMT), the regulation of cancer stem cells (CSCs), the development of tumor invasion and migration. miRNA also acts as a biomarker stably expressed in serum and provides new target for molecular target therapy of various cancers. The aim of this review is to illustrate the new role of miRNA in carcinogenesis and highlight the new prospects of miRNA in cancer clinical application, such as in serological diagnosis and molecular-targeted therapeutics.

Keywords microRNA; carcinogenesis; epithelial–mesenchymal transition; cancer stem cells; invasion and migration; cancer clinical application

Received: March 18, 2011 Accepted: May 27, 2011

Introduction

In recent years, microRNA (miRNA) has been intensively studied as ‘star molecule’. It is a cluster of small non-encoding RNA molecules of 21–23 nucleotides in length. miRNA gene is first transcribed to produce molecule hundreds of nucleotides in length by RNA polymerase II, which is called pri-miRNA. Then, RNA polymerase III, also called Drosha, processes pri-miRNA into a stem-loop structure that has 60–70 nucleotides in length, which is called pre-miRNA. The pre-miRNA is exported from the nucleus into cytoplasm by transportation protein, Exportin-5, in a Ran-GTP-dependent manner [1]. With the involvement of another RNA polymerase III, Dicer, the pre-miRNA is further cleaved into double-stranded RNA (dsRNA). Then one strand of the dsRNA is degraded by a helicase, leaving the other strand, a mature active miRNA single strand, to enter the nucleus and form the miRNA-associated RNA-induced silencing complex (miRISC) [2,3]. With this complex, miRNA can play an essential role in biological functions.

miRNA controls the expression of target gene at the post-transcriptional level by inhibiting protein translation or degrading cognate target mRNA through binding to its 3′-untranslated region (3′-UTR) or coding sequence (CDS) with varying degrees of sequence complementarity [4]. The 3′-UTR of target mRNA owns miRNA recognition elements [5]. Meanwhile, through a critical region called ‘seed region’, which includes 2–8 nucleotides from 5′-end of miRNA, the mature miRNA could bind to the target mRNA in 3′-UTR [6–9].

Most plant miRNA is perfectly complementary with its target mRNA coding sequence (CDS) or open reading frame, and fully degrades its target mRNA. However, the miRNA in majority of animals is imperfectly complementary with the 3′-UTR of its mRNA, and so the post-transcriptional translation is inhibited rather than completely blocked [10,11]. Based on this regulatory mechanism, miRNA wins widespread attention on its potential role of post-transcriptional regulation and its exertion of multiple biological functions [12].

Nowadays, miRNA becomes an essential player in cancer research, the abnormality of miRNA expression will directly affect the exertion of target gene function, as it can induce or prevent tumor progression. Based on miRNA target, molecular target therapy will become the most effective method in cancer therapeutic field. Meanwhile, using miRNA as a biomarker and testing the expression level of miRNA in serum will also give us new view on tumor diagnosis and treatment. So in this review, we mainly illustrated new role of miRNA in carcinogenesis, indicated its clinical application in serological diagnosis

and molecular-targeted therapeutics, and further summarized their relationships and future prospects.

New Role of miRNA in Carcinogenesis

miRNA and epithelial−mesenchymal transition

Epithelial−mesenchymal transition (EMT) is a regulation process of epithelial and mesenchymal cell phenotype, which means that cell phenotype is not static but highly dynamic instead [13]. The inter-conversion between epithelial and mesenchymal cell phenotype can be termed as EMT, and the reverse process can be named as mesenchymal−epithelial transition (MET) [14].

EMT begins when the epithelial cell loses apico-basal polarity and its tight junctions dissolve; meanwhile, the cell surface protein like E-cadherin is replaced by vimentin, which is a mesenchymal phenotype marker [15]. In the EMT process, the epithelial markers, particularly E-cadherin, is down-regulated. However, the mesenchymal markers, particularly vimentin or fibronectin, are up-regulated. Additionally, EMT increases cell migration and invasion capacities, and spreads blood or lymph metastasis, and so it represents an essential mechanism for tumor metastasis and progression [16].

EMT is also a crucial regulatory process of cell growth and metastasis, in which the non-encoding RNA acts as the key regulator. In bladder cancer cell lines, the miR-200 family, including miR-200a/b/c, miR-141, miR-429, and miR-205, expresses the 'epithelial' phenotype. Meanwhile, four epithelial cell lines, including UMUC6, UMUC9, UMUC16, UMUC5 [E-cadherin(+)], five mesenchymal cell lines, including UMUC2, T24, UMUC3, UMUC13, KU7 [E-cadherin(−)], and one epithelial/mesenchymal cell line:253J BV are all mentioned in bladder cancer cell lines [17]. The miR-200 family has the E-cadherin-positive phenotype and is always up-regulated in epithelial cells. While in mesenchymal cells, the expression levels of miR-200 family are down-regulated [18].

Further research has confirmed that the expression levels of miR-200 family negatively correlate with the expression level of Zinc finger E-box-binding homeobox 1 (ZEB1) and Zinc finger E-box-binding homeobox 2 (ZEB2), which are both target genes of miR-200 family in human breast cancer [19,20]. ZEB1 and ZEB2 also act as inhibitors of E-cadherin expression, and negatively correlate with the expression level of E-cadherin [21]. In mesenchymal-like ovarian cancer cells, the over-expression of miR-429, which also belongs to the miR-200 family will lead to the reversal of mesenchymal phenotype and make the MET [22]. Meanwhile, in the diagnosis and treatment of ovarian cancer, miR-429 is not only of great use for a biomarker of EMT, but also of great therapeutic value against cancer metastasis and recurrence [23]. So it can be concluded that the miR-200 family is a crucial regulator in EMT of various cancers, the epithelial phenotype is under the regulation of miR-200 family, whose expression will finally induce the emergence of 'mesenchymal−epithelial transition' phenotype.

Other studies have further shown that in human fetal pancreatic epithelial cells, the miR-30 family closely relates to EMT. Using anti-miRs to deplete the expression of miR-30 family will lead the epithelial phenotype in transition to mesenchymal phenotype, whereas the over-expression of miR-30 family will keep the epithelial phenotype maintenance [24]. Further research has revealed that the mesenchymal mRNAs, such as vimentin and snail-1, whose translations can be inhibited by miR-30 family, are both targets of the miR-30 family [25]. In summary, the concept of EMT and its reverse process MET, together with their related miRNAs, will form a fundamental framework for us to understand more about carcinogenesis, and will likely provide new therapeutic target for tumor invasion and metastasis.

miRNA and cancer stem cells

Stem cells can be divided into two types, normal tissue stem cells and cancer stem cells (CSCs). Both normal stem cells and CSCs, which largely share surface marker phenotype and molecular machinery, are generally defined by their potential for multilineage cell differentiation and capability of self-renewal [26]. It is generally believed that CSCs or tumor-initiating cells (TICs) usually arise from normal stem cells, but with enhanced self-renewal capacities, and possess stem cell-like properties such as tumorigenic capacity, serial passage, expression of unique surface markers which allow for reliable identification and purification [27,28].

Meanwhile, several signaling pathways and molecules are indicated to modulate survival, self-renewal, and differentiation of CSCs, such as Hedgehog, Notch, Wnt/β-catenin, HMGA2, Bcl-2, Bmi-1, c-Myc, and c-Met [29–31]. Among them, Hedgehog pathway plays a key role in stem cell maintenance and dysregulation of self-renewal in CSCs [32]. Through inhibition of the Hedgehog pathway, CSC-targeted treatment will become a promising therapeutic strategy when combined with conventional cytotoxic agents [33−36].

Recent discoveries have confirmed that CSCs closely relate to miRNA. Through affecting expression level of target gene and protein in cell proliferation and cell death, miRNA could regulate initiation and progression of tumor [37,38]. The miR-34 family is directly under the regulation of p53, and plays a role as tumor suppressor-like p53 in p53 deficient human pancreatic cancer cells. Their target genes, such as Notch and Bcl-2, are both involved in survival and self-renewal of CSCs [39]. Restoration of the

miR-34 will inhibit pancreatic cancer cells cloning proliferation but activate caspase-3 so as to induce cell apoptosis [40]. Meanwhile, miR-34 could increase chemotherapy and radiotherapy sensitivities of cancer cells [41].

Most importantly, restoration of miR-34 will inhibit tumor sphere growth *in vitro* and tumor formation *in vivo*. Meanwhile, it could also inhibit the growth of TICs or CSCs with biomarkers of CD44+/CD133+, which own high expression of Bcl-2, but deficient in miR-34 expression [42,43]. Further research has confirmed that miR-34 is essential in pancreatic CSCs' self-renewal by directly regulating downstream target gene Notch and Bcl-2 [44]. Through regulating CSCs and inducing restoration of miR-34 (which could act as a tumor suppressor), some new therapeutic methods for pancreatic cancer patients with p53 deficiency may be developed [45].

Additional findings have revealed that TGF-β could up-regulate the expression of miR-181 at the post-transcriptional level, meanwhile. Through interfering with tumor suppressor Ataxia telangiectasia mutated (ATM), a target gene of miR-181, TGF-β pathway could regulate the properties of CSCs [46]. It provides a totally new relationship between miRNA and CSCs. Using miRNA to regulate the properties of CSCs will definitely bring new hope for CSC-targeted therapies. Other studies have confirmed that side population (SP) cancer cells are a minor population of cancer cells that can be identified in various tumors and own properties of CSCs. In SP cancer cells, the expression level of miR-21 accompanied with its upstream regulator activator protein-1 (AP-1), is usually found to be up-regulated. miR-21 plays a key role in maintaining chemo-resistant characteristics of SP cells [47]. Meanwhile, for the impairment of resistance to chemotherapy in CSCs, miR-21 will be a promising target in molecular target therapies.

Another research has demonstrated that let-7 family is down-regulated in several cancers and acts as tumor suppressors and regulators of cell differentiation and apoptosis. The let-7 family closely relates to CSCs, in which the levels of let-7 family are low, and so they own unlimited self-renewal capabilities and promote metastasis and cancer progression [48]. In breast cancer, RAt Sarcoma (RAS) and high-mobility group AT-hook 2 (HMGA2) are found to be let-7 targets, the low levels of let-7 in stem cells inversely correlate with the high HMGA2 and RAS expression. RAS and HMGA2 regulate different aspects of stemness. RAS is important for self-renewal; HMGA2 on the other hand seems to help in maintaining stem cell multipotency. So the over-expression of RAS and HMGA2 in breast cancer will lead to poor prognosis [49]. In conclusion, doing further research between miRNA and CSCs will not only help us know more about mechanisms and biogenesis of CSCs in carcinogenesis, but also help us find more therapeutic targets for tumor diagnosis and treatment.

miRNA and tumor invasion and migration

Tumor invasion and migration are multi-step processes that can be defined as tumor cells spread from primary lesions to another non-adjacent organs or tissues to form secondary tumor lesions through blood dissemination, lymph, or distant metastasis [50]. Tumor metastasis takes place in a series of pathological processes: vascularization progresses and the primary tumor grows, then cancer cells detach and invade into lymphatic and blood vessels, surviving, and arresting in circulation; further they extravasate into new microenvironments and finally colonize and grow into metastatic tumors [51,52].

Meanwhile, there are some physiological processes and small molecules which are essential for tumor metastasis, such as EMT, MET, vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs). They not only survive the growth of metastatic tumor colonies, but also affect cancer cells intravasation and extravasation [53]. In other words, all the processes, including the activation of MMPs, the breakdown of extracellular matrix, the emergence of EMT, the establishment of microcirculation, and the increase of cell motility and invasiveness, will work together to induce the beginning of tumor invasion and migration [54,55].

Recent findings have demonstrated that miRNA is involved in the regulation of various stages of invasion and migration, in which miRNA acts as either the activator or the suppressor of metastasis [56]. miR-9 is a MYC/MYCN activated miRNA, the expression of which is up-regulated in breast cancer. Cadherin-1 (CDH1), the target of miR-9, is an E-cadherin-encoded messenger RNA, which could increase cell mobility and invasiveness capabilities [57]. miR-9 mediates down-regulation of the E-cadherin level, activates the β-catenin signaling pathway, and further up-regulates the expression level of VEGF in order to induce tumor angiogenesis [58]. Down-regulation of E-cadherin and activation of β-catenin are necessary but not sufficient condition for miR-9-dependent up-regulation of VEGF. However, E-cadherin is still a key target of miR-9 regulation [59]. All the studies above have indicated that miR-9 acts as a tumor initial miRNA, which is involved in the regulation of the signaling transduction pathway and directly controls the expression level of E-cadherin, a key protein in tumor metastasis suppression. miR-9 could also induce tumor angiogenesis, EMT and the formation of micro-metastasis [60,61]. So the inhibition of miR-9 expression will suppress the formation of tumor metastasis.

Other research has revealed that in oral squamous cell carcinoma, the over-expression of miR-181 will enhance tumor capabilities of migration and invasiveness.

Meanwhile, it closely relates to vascular invasion, lymph-node metastasis, and poor prognosis [62]. Additional studies have revealed that in prostate cancer; systemically delivery of miR-34a will inhibit tumor invasion and metastasis, and extend the lifespans of tumor-bearing mice [63]. Further findings have identified that CD44 is a direct and functional target of miR-34a, and over-expression of miR-34a or knockdown of CD44 will inhibit metastasis and recurrence of prostate cancer [43].

Additional discoveries have shown that in breast cancer, knockdown of miR-340 expression will lead to tumor invasion and metastasis, while induction of miR-340 expression could effectively suppress tumor invasion and migration. In addition, loss of miR-340 expression will contribute to lymph node metastasis, high tumor histological grades, poor clinical stages, and shorter overall lifespans of breast cancer patients. It has also been identified that c-Met is a direct target of miR-340, which could modulate cell invasion and metastasis through regulating the expression of MMP-2 and MMP-9 [64]. To sums up, it is undoubted that miRNA plays a key role in tumor invasion and migration processes [65,66]. Meanwhile, doing further researches on the relationships between miRNA and tumor metastasis will not only bring a new direction for us to study biological mechanisms of tumor invasion and metastasis, but will also provide a new target for early diagnosis and treatment of multiple cancers.

New Prospects of miRNA in Cancer Clinical Application

miRNA and serological diagnosis

Circulating miRNA or cell-free miRNA stably exists in serum or body fluids. They not only contain sufficiently stable miRNA signatures for diagnosis or prognosis of cancer, but also affect essential biological processes in cancer initiation and progression. So they can be used as non-invasive biomarkers for early diagnosis of different tumors [67,68]. The alteration of circulating miRNAs in serum may reflect and indicate physiological or pathological changes in multiple cancers, and they may also be used as crucial surrogate biomarkers [69]. Additionally, circulating miRNAs closely relate to tumor progression or metastasis, and so they can act as valuable prognostic markers to predict clinical outcomes of patients suffering from cancer [70–74].

Dysregulation of miRNAs in serum or body fluids closely relate to various cancers. The expression of circulating miRNAs in serum is stable, not easily degradable [75]. When compared with exogenous synthetic miRNAs, endogenous cell-free miRNAs exist in serum in form of RNase-resistant types and own characteristics of self-renewable, resistance to DNase/RNase digestion and repeated frozen [76]. Meanwhile, endogenous cell-free miRNAs can also resist to other bad environments, such as boiling, low/high pH. Moreover, the expression levels of endogenous cell-free miRNAs in the same population are consistent [77].

Based on the principles above, circulating miRNAs can be used as specific biomarkers for various tumors, as it is a convenient non-invasive test method to diagnose tumors in early stages [78,79]. For example, in lung cancer, the serological-specific miRNAs include miR-205, miR-206, miR-335, miR-1254, and miR-574–5p [80]; in colorectal cancer, the serological-specific miRNAs include miR-485–5p, miR-361–3p, miR-326, and miR-487b [81,82]; in diffuse large B cell lymphoma, the serological-specific miRNA includes miR-21 [83]. Moreover, in leukemia, patients are shown to have specific circulating miR-92a down-regulated [84]; in metastatic prostate cancer, circulating miR-141 has been significantly elevated in serum [85,86]; in pancreatic cancer, miR-21, miR-155, miR-196a, and miR-210 are all found to be up-regulated in plasma, and miR-196a is confirmed as a new prognostic marker for pancreatic cancer [87–89].

Taken together, serological diagnosis of tumor-specific circulating miRNA will be a promising approach for early diagnosis and treatment of cancer in a non-invasive manner. Meanwhile, this new diagnosis method will surely bring dramatic changes for clinical rating, prognosis judging, effect forecasting, and recurrence monitoring of various cancers.

miRNA and molecular target therapy

There are two strategies of molecular therapy targeted at miRNA, one being miRNA reduction, and the other being miRNA over-expression. In the former strategy, we use anti-miRNA oligonucleotides (AMOs) to directly compete with the interactions of endogenous miRNAs and its target genes [90]. Sometimes, AMOs have to be modified on their 5′ end, by adding 2′-Omethyl and 2′-O-methoxyethyl groups, in order to improve their effectiveness and stability [91]. Meanwhile, the locked-nucleic-acid antisense oligonucleotides exhibit relatively low toxicity and they can also be designed to reduce target miRNAs expression level [92].

Moreover, using synthetic mRNAs, which contain multiple binding sites for endogenous miRNAs and effectively repress expression levels of miRNA families that share the same seed sequence, miRNA sponges and miR-masking can also be used to reduce interactions between miRNAs and their targets [93]. Additionally, using small-molecule inhibitors against specific miRNAs will be another promising strategy to increase efficiency and specificity of miRNA reduction [94].

In the later strategy, using viral or liposomal delivery systems, the expression levels of miRNAs, which own

tumor-suppressive characteristics, can be elevated and they can restore tumor inhibitory functions in cancer cells [95,96]. Meanwhile, miRNA mimics, which are small, chemically modified double-stranded RNA molecules designed to mimic endogenous mature miRNAs can also be used as promising tools to increase the target miRNAs' expression level [97].

Based on the theories above, using miRNA as a target for molecular therapy, it will become a new direction for cancer treatment. Specific blocking or inducing miRNA-mediated biological processes could reverse the development of cancers [98]. Introducing oncogenic complementary oligonucleotides of miRNA will effectively decrease the expression of miRNA in tumors and delay tumor growth. Conversely, high expression of miRNA, which owns capability of tumor suppressor, can be used to treat some specific tumors [99].

Besides, while some miRNAs aim at self-target molecular therapies, there are other miRNAs which can mediate molecular target drugs to play effective roles in anti-cancer therapies. For example, in bladder cancer, inhibition of epithelial growth factor receptor (EGFR), VEGFR, FGFR, and PDGFR, is becoming an attractive therapy strategy [100]. Through exploring the relationship between expression of miRNA-regulated target genes and their reactions to drug therapies, it has been found that the miR-200 family could reverse 'EGFR-resistant' phenotype, down-regulate expression of ERRFI-1, and further modulate EMT and increase the sensitivities of anti-EGFR therapies [17].

Moreover, some discoveries have revealed that in human and murine squamous cell carcinomas, there exists an miRNA-dependent regulation of p63/p73 crosstalk, which could modulate p53-independent survival [101]. Additionally, p63 could suppress the expression level of miR-193a-5p, while the proapoptotic p73 isoforms in both normal cells and tumor cells could enhance miR-193a-5p expression [102]. Chemotherapy will contribute to p63/p73-dependent induction of miR-193a-5p. Due to this miRNA-mediated feedback inhibition of p73, chemosensitivity of this cancer can be limited [103,104].

Essentially, the inhibition of miR-193a expression will disturb this feedback and therefore repress viabilities of cancer cells and greatly increase chemosensitivities of this kind of tumor. In consequence, this direct and miRNA-dependent regulatory network is forming to regulate chemosensitivities and chemoresistances of this cancer. Meanwhile, p53, p63, and p73-related miRNA will provide a new potential target for molecular target therapies of p53-deficient tumors [105,106].

Additionally, latest findings have shown that the core of blocking metastasis-related miRNA will definitely bring a novel direction for tumor metastasis-related molecular-targeted treatment [107]. Another innovative study confirmed that the expression level of target miRNA can be effectively down-regulated through a lentiviral vector ('anti-miRNA decoy') by targeting multiple complementary binding sites of target miRNA [108]. In a similar approach, the so-called miRNA mimics can also be

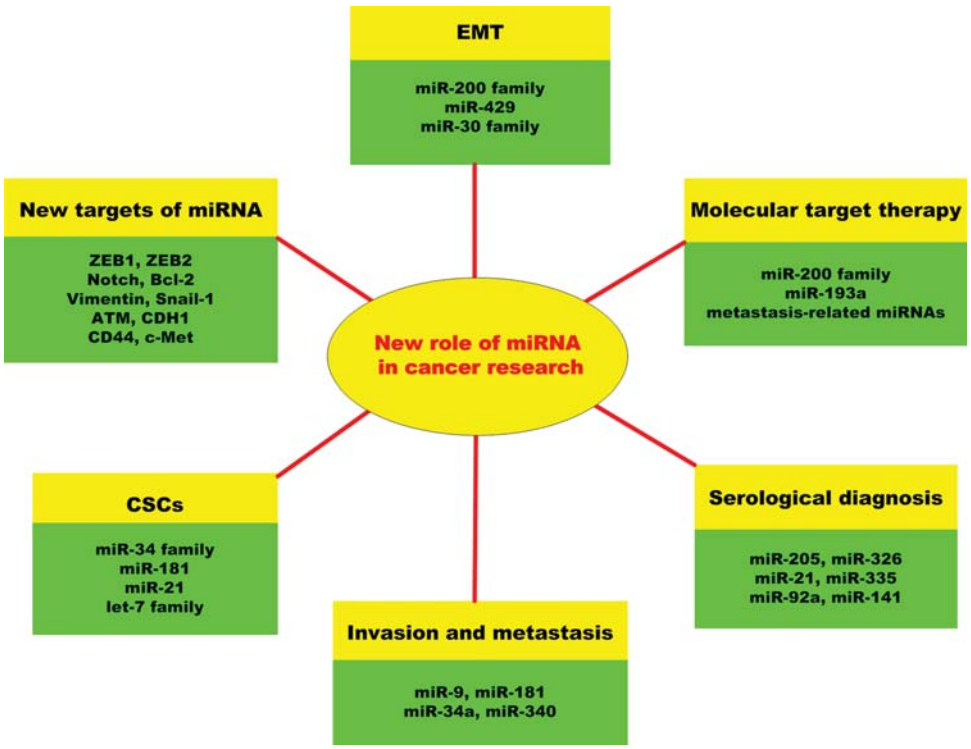

Figure 1 New role of miRNA in cancer research

Table 1 Biological functions and relationships between miRNA and its targets

miRNA	Types of cancer	Targets	Biological functions	Expression level	References
miR-200 family	Bladder cancer Breast cancer Ovarian cancer	ZEB1 and ZEB2	EMT MET	↓[a]	[17,22]
miR-30 family	Pancreatic cancer	Vimentin and snail-1	EMT	↓	[25]
miR-34 family	Pancreatic cancer	Notch and Bcl-2	Involved in the self-renewal of CSCs	↓	[39]
miR-181	Breast cancer	ATM	Regulate the properties of CSCs	↓	[46]
let-7 family	Breast cancer	RAS and HMGA2	Regulate stem cells differentiation and self-renewal capabilities	↓	[48,49]
miR-9	Breast cancer	CDH1	Induce tumor angiogenesis, EMT and metastasis	↑[a]	[57]
miR-34a	Prostate cancer	CD44	Induce tumor invasion and metastasis	↓	[43]
miR-340	Breast cancer	c-Met	Lead to tumor invasion and migration	↓	[64]

[a]↑ and ↓ indicate that the expression level of miRNA is up-regulated and down-regulated in corresponding cancer, respectively.

introduced to substitute for pathologically down-regulated miRNA in various tumors, which is called the 'miRNA replacement therapy' [109,110].

In other words, the molecular target therapy of miRNA in cancer will certainly bring a promising direction for us to do more researches. We are sure that more target miRNAs will be found, more advanced technologies will be effectively used, and more tumor patients will get benefit from this kind of novel therapeutic method.

Conclusions and Perspectives

From all the studies illustrated above, we know that miRNA plays a key role in carcinogenesis, and it is also involved in the process of EMT, the regulation of CSCs and the development of tumor invasion and migration. Meanwhile, circulating miRNA in serum can be used as non-invasive biomarker for diagnosis of various cancers. Moreover, the molecular-targeted therapy of miRNA will become a promising method for us to deal with malignancies in a more specific and an efficient manner. So, we can draw a figure to describe new role of miRNA in cancer research (**Fig. 1**). We can also make a table to show biological functions and relationships between miRNA and its targets (**Table 1**).

Nowadays, miRNA is becoming a hot issue in medical research field. The researches focused on miRNA have expanded from oncology field to more complicated pathogenesis studying field. We are now seeking specific miRNA of various cancers and diseases in order to achieve early diagnosis and treatment. Although we have already found the locations of multiple target genes of miRNA and understood their biological functions, it is only 'a tip of the iceberg', there are still more pathogenesis, carcinogenesis, and biological mechanisms on miRNA need to be studied.

Meanwhile, lots of problems based on new role of miRNA are emerging, such as how to understand the role of miRNA and its interactions in the processes of EMT, in the regulation of CSCs self-renewal and in the development of tumor invasion and metastasis. How do these three processes affect the progression of each other and how do they consist of a complex network in order to have the co-effect of miRNA expression? Are there some highly specific miRNAs existing in serum, which are involved in both EMT and CSCs self-renewal processes, so that they can be used as indicators of tumor invasion and progression? Are there some more efficient molecular target therapies existing in the cancer therapeutic field so as to effectively deal with tumors when combined with traditional chemotherapy, radiotherapy, and other anti-cancer drugs?

Additionally, more advanced methods for diagnosis and treatment of cancer based on miRNA need to be explored and perfected. In other words, we have reasons to believe that, with the in-depth study of miRNA, it will definitely lead us to a totally new world of life sciences, will inject fresh vitality to the development of medicine and will finally bring a new gospel for more patients.

Funding

This work was supported by the grants from the State Key Basic Research and Development Plan of the Ministry of Science and Technology of China (2011CB504300) and the Key Project of National Natural Science Foundation of China (30930101).

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