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Review Article

MicroRNA Function in Human Diseases

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Abstract
MicroRNAs are emerging as a hot topic in research, and rightfully so. They show great promise as targets of treatment and as markers for common human diseases, such as cancer and metabolic diseases. In this review, we address some of the basic questions regarding microRNA function in human disease and the clinical significance of microRNAs. Specifically, microRNAs in epigenetics, cancer, and metabolic diseases are discussed, with examples taken from cholangiocarcinoma and nonalcoholic fatty liver disease.

Introduction

MicroRNAs are small, endogenously expressed, single-stranded, noncoding RNAs about 19–24 nucleotides in length [1]. Found throughout the human genome, microRNAs work to fine-tune gene expression [2, 3]. This leads to the following question: are microRNAs epigenetic? Many factors need to be carefully considered before answering this question. What we know for certain is that microRNAs, or more specifically their aberrant expression, are linked to all human cancers. The current review specifically looks at the aberrant expression of a multitude of microRNAs implicated in cholangiocarcinoma, tabulating microRNAs known to be dysregulated in malignant cells and tumors, as compared to normal cells and tissue. Additionally, microRNAs play a major regulatory role in metabolic diseases, such as nonalcoholic fatty liver disease. Much like in cancer, an aberrant expression of microRNAs has been reported in patients with metabolic disease. Since microRNAs can be

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easily found in tissue and biological fluids such as serum [4], cerebrospinal fluid [5], aqueous humor [6], milk [7], bile [8], peritoneal fluid [7], ovarian follicular fluid [9], and urine [10], the measurement of microRNA expression levels can be used to detect diseases. In addition to disease detection, successful treatment can result in a return to normal levels of microRNAs, suggesting a role in disease monitoring and prognosis. Unfortunately, we cannot provide a comprehensive review of the state of microRNA research, due in no small part to the rapid proliferation of microRNA studies; we hope to provide a few illustrative examples because microRNAs will have an important role in both the diagnosis and treatment of human diseases.

MicroRNA Function

MicroRNAs are expressed from a dedicated gene (termed intergenic with reference to protein-coding genes) or are processed from the RNA of a host gene (whereby both the mRNA and microRNA are products of the same primary transcript). The mature microRNA sequence can be derived from either introns or exons. MicroRNAs can be grouped into larger microRNA families which share conserved sequences. Within the genome, microRNAs are found clustered together or single, and clusters can contain related family members or disparate microRNAs. Once expressed, microRNAs are incorporated into the RNA-induced silencing complex and direct repression of a target mRNA by base complementarity within a 6- to 8-nucleotide sequence, the 'seed' sequence, of the microRNA [11, 12]. This leads to either degradation or repression of the target mRNA, impacting proliferation, apoptosis, and differentiation of cells. MicroRNAs act on several oncogenes or tumor suppressors, contributing to cancer formation and progression. Importantly, aberrant expression of microRNAs has been linked to all human cancers. In addition to cancer biology, microRNAs target a high proportion of cellular mRNAs (estimated as high as 60% of genes) and can impact normal physiology and nonmalignant disease. On the clinical level, microRNAs are surfacing as a novel diagnostic tool for the early detection, classification, and perhaps treatment of human disease.

MicroRNAs and Epigenetics

Should gene regulation by microRNAs always be considered an epigenetic process? There is controversy concerning the exact definition of epigenetics and whether it is necessary for an epigenetic effect to be heritable through either a meiotic or mitotic cell division [13, 14]. Indeed, there are arguably cases where the same molecular change in one circumstance is heritable and in another is transient. Fittingly, there is also difficulty in determining if the effects of microRNAs to fine-tune gene expression should be considered epigenetic. If the heritability standard is enforced, then microRNAs would generally be considered regulatory. However, note that in the absence of microRNAs, the development of the zebrafish is impaired (e.g., gastrulation, brain formation, somitogenesis, and heart development), but injection of miR-430 into the single-cell fertilized embryo resulted in phenotypic rescue of these later-stage processes. Further, the model embryos had to be deficient in microRNAs *and* derived from an egg lacking microRNAs (maternal-zygotic deletion), because the maternal contribution was sufficient to allow development. The rescue of later-stage developmental processes by either maternally derived or injected miR-430 is consistent with this microRNA acting in daughter cells after several rounds of division, for example a heritable effect [15]. This does not indicate that miR-430 expression was self-reinforcing, but only that some microRNAs are stable enough to maintain a functional concentration in a daughter cell even after numerous cell divisions. To complicate matters, microRNAs are regulated by classic imprinting [reviewed

in 16–18] and can target proteins mechanistically involved in epigenetic DNA methylation, for example DNMT3 and DNMT1 [19, 20] and methyl CpG binding protein 2 [21]. Thus, it may be better to ask whether a *specific* microRNA is acting in an epigenetic manner rather than broadly trying to classify all microRNA effects as epigenetic.

MicroRNAs in Cancer

Early in the study of microRNAs in human disease, it became clear that microRNAs were involved in cancer. For instance, a minimally deleted region of chromosome 13 in chronic lymphocytic leukemia contained the related microRNAs, miR-15a and miR-16-1, and these microRNAs were lost or decreased in cancerous cells [22]. Separately, the analysis of small RNAs cloned and sequenced from colorectal adenocarcinoma tissues revealed decreased levels of miR-143 and miR-145, with levels of both reduced versus nontumor colonic tissues [23]. Since these early studies, there has been a marked expansion in microRNA research. Altered microRNA expression is now well recognized in human malignancies, including examples where microRNAs are expressed at abnormally high levels in tumor cells or at significantly reduced levels. Because there is far more information on microRNAs in cancer than can be covered in a single short review, we have chosen to discuss microRNAs in the biliary tract cancer cholangiocarcinoma, an area of active research in our lab, as an illustrative example.

MicroRNAs in Cholangiocarcinoma

Cholangiocarcinoma is a relatively rare but highly malignant cancer of the biliary tree that shares a number of features with other cancers, including the risk factors for chronic inflammation and injury [24]. Aberrant microRNA expression has been implicated in cholangiocarcinoma progression, though our understanding is still incomplete. Table 1 lists 47 microRNAs whose expression is increased in cholangiocarcinoma. It is organized to group microRNAs from the same family that share sequence similarity, and presumably function, with other family members. Not all members of the family are included in table 1, but only those with altered levels in disease. The 47 increased microRNAs represent 34 microRNA families. On the other hand, 53 microRNAs are decreased in cholangiocarcinoma (table 2) that belong to 42 microRNA families. There is some overlap, with 5 microRNAs, miR-22-3p, miR-122-5p, miR-200c-3p, miR-221-3p, and miR-424-5p, having been measured at increased and decreased levels in different studies. Whether this reflects differences in disease etiology, stage, or behavior is not yet known. Note that the expression changes – either up or down – have been linked with a functional target in the minority of cases. Studies in other tumor types as well as future studies in cholangiocarcinoma will help determine the functional role of each of the altered microRNAs.

MicroRNAs in Metabolic Disease

Metabolic syndrome is defined as three or more of the following: abdominal obesity, hypertriglyceridemia, low HDL cholesterol, hypertension, and elevated blood glucose. Metabolic syndrome has an estimated prevalence of up to 34% of all adults in the USA [25]. Diseases associated with metabolic disease, including obesity, coronary artery disease, type 2 diabetes, nonalcoholic fatty liver disease, and polycystic ovarian syndrome, each have been reported

Table 1. Upregulated microRNAs in cholangiocarcinoma

miR family	miR members	Targets/functions	Ref. No.
8	141-3p/200b-3p/200c-3p/429	apoptosis (200b-3p)	57, 63, 74
10	10a-5p		74
15	15a-5p/15b-5p		61
17	17-5p/17-3p/20a-5p/20b-5p/93-5p/106a-5p/106b-5p		61, 62, 64, 74
19	19a-3p		61, 62
21	21-5p	PTEN, PDCD4, TIMP3, invasion, metastasis, tumor growth, apoptosis	57, 61, 62, 65, 66, 71–74
22	22-3p		74
24	24-3p		74
25	25-3p	DR4, apoptosis	61, 62, 64
26	26a-5p	GSK-3β, proliferation	68
27	27a-3p		74
28	151a-3p		74
29	29a-3p/29b-3p		74
30	30b-5p/30e-5p		74
96	96-5p		74
103	103a-3p/107		61, 74
122	122-5p		74
130	130b-3p		61
135	135b-5p		74
142	142-3p		61
181	181a-5p		74
192	192-5p		63
193	193a-3p		61
203	203a		74
221	221-3p		74
223	223-3p		61, 72
224	224-5p		61
322	424-5p		74
324	324-5p		61
331	331-3p		61
340	340-5p		74
374	374a-5p		61
663	663b		74

MicroRNAs found to be increased in human cholangiocarcinoma cells or tissues are listed by family, with the particular altered microRNA listed in the 'members' column. When studied, the function and/or target protein(s) are included.

to have altered microRNA expression and function [9, 26–36]. MicroRNAs are involved in a number of metabolic processes, such as maintenance of cellular glucose, cholesterol, triglyceride, and fatty acid metabolism [37].

A pathologic contribution of microRNAs to metabolic syndrome seems likely, including a role in inflammation. For example, decreased expression of miR-132 and miR-155 in adipose tissue is associated with increased expression of the proinflammatory cytokine IL-6 in obese

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Table 2. Downregulated microRNAs in cholangiocarcinoma

miR family	miR members	Targets/functions	Ref. No.
1	1		74
let-7	let-7a-5p/let-7b-5p/let7c/98-5p	NF2 (let-7a-5p), apoptosis	58, 61, 74
8	200c-3p	NCAM1, epithelial-mesenchymal transition	61, 70, 72
29	29b-3p	Mcl-1, apoptosis	59
22	22-3p		63
31	31-5p		72
99	99a-5p/100-5p		74
122	122-5p		72
124	124-3p	SMYD3, migration and invasion	69
125	125a-5p/125b-5p		63, 74
126	126-3p		74
127	127-3p		63, 74
139	139-3p		74
144	144-3p		74
145	145-5p		61, 72, 74
146	146a-5p		72
148	148a-3p/152	DNMT1, proliferation	20
154	494	PTTG1, TOP2A, cell cycle progression	62, 67
184	184		61
185	185-5p		61
188	188-5p		62
197	197-3p		61
191	191-3p		62
198	198		61, 62
199	199a-5p		63
204	204-5p		61
214	214-3p	Twist, epithelial-mesenchymal transition	61, 63, 66
221	221-3p/222-3p		61, 72
290	371a-3p		61
302	302b-3p/302d-3p		61
320	320a		61
322	424-5p		63
328	328		61
337	337-3p		61, 74
338	338-3p		61
368	376a-3p		63, 74
370	370	MAP3K8, proliferation	60, 62
373	373-3p		61
451	451a		74
506	512-3p/513a-5p		62
515	517c-3p/519a-3p/520e		62, 74
630	630		74
662	662		62

MicroRNAs that were decreased in cholangiocarcinoma cells or tissues are grouped by family, and individually altered microRNAs are listed as miR members. If known, the relevant target proteins or functions in cholangiocarcinoma are included.

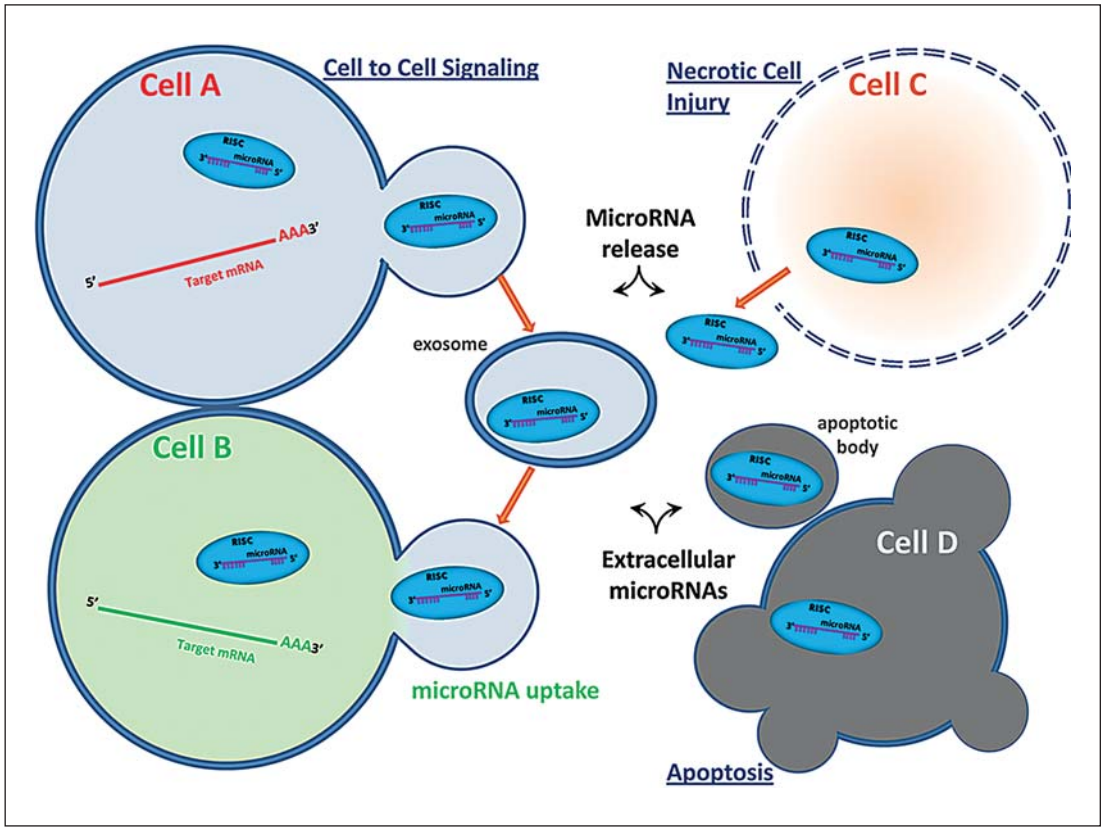

Fig. 1. MicroRNAs are released from cells. MicroRNAs have been detected in many extracellular fluids, and are potentially released by regulated processes such as exosome-mediated release or as part of lipid particles (not shown). Alternatively, injury (either necrotic or apoptotic) can result in the release of intracellular microRNAs into the extracellular compartment. Note that endogenous microRNAs detected in biological fluids are consistently more stable than exogenous microRNAs spiked into the same sample. This is consistent with protection of endogenous microRNAs in ribonucleoprotein complexes, in membrane-bound vesicles, or in another stabilizing complex. RISC = RNA-induced silencing complex.

individuals [38]; studies in patients with nonalcoholic fatty liver disease have revealed a similar increase in hepatic and circulating levels of IL-6 [39, 40]. For additional information regarding altered expression of microRNAs in adipocyte differentiation, inflammation, adipogenesis, and insulin signaling in obese individuals, we refer the reader to excellent recent reviews [38, 41]. Here, we will further discuss microRNA signaling in nonalcoholic fatty liver disease.

Altered MicroRNA Levels in Nonalcoholic Fatty Liver Disease

Nonalcoholic fatty liver disease is a spectrum of liver disease including simple steatosis, nonalcoholic steatohepatitis (NASH), advanced hepatic fibrosis, liver cirrhosis, and hepatocellular carcinoma [42]. Aberrant expression of various microRNAs has been reported in patients with NASH [26, 43]. NASH patients, for example, had elevated levels of miR-34a and miR-146 and decreased levels of miR-122 in the liver [26], and increasing miR-34a expression in the liver was found to correlate with increasing severity of NASH [44, 45]. Free fatty acid-

induced lipotoxicity is due, in part, to an increase in miR-34a expression in hepatocytes resulting in repression of the antiapoptotic protein, Sirt1 (a protein deacetylase). Decreased Sirt1 expression caused by miR-34a results in an increase in acetylation of p53 and activates the expression of p53 targets such as PUMA, a proapoptotic BH3-containing protein, promoting the induction of hepatocyte lipoapoptosis [44]. Indeed, PUMA dysregulation appears to be important in nonalcoholic fatty liver disease, as levels of PUMA mRNA and protein were elevated in patients with NASH compared with patients with simple steatosis and control population [46]. Separately, PUMA expression in nonalcoholic fatty liver disease was controlled by miR-296-5p, which was downregulated in patients with NASH [47].

MicroRNA Measurement in Disease

MicroRNAs can be detected in diseased tissue as well as in biological fluids. Some of the mechanisms of microRNA release from the cell of origin are diagrammed in figure 1 [48–52], and microRNAs can communicate between cells [53]. Thus, the measurement of altered microRNAs can be performed from tissue biopsies or from relevant biological fluids. In the latter case, detection of microRNAs in biological fluids still leaves a question as to their cell of origin [54]. If the diseased tissue is releasing more microRNAs, it may be a reflection of cellular injury or increased synthesis and export. If another cellular source is responsible, the increased extracellular microRNA may reflect injury to a secondary tissue or recruitment of an inflammatory cell, for instance. This ambiguity should be considered when altered microRNAs in fluids are observed, but also may allow for the detection of related pathologic processes.

In addition to a correlation between microRNA expression and disease, successful treatment can result in a return to normal microRNA levels. In the case of obesity, for example, the circulating levels of miR-140-5p and miR-142-3p progressively increased in nonobese (BMI <30) to obese (BMI 30–40) to morbidly obese (BMI >40) patients. After bariatric surgery, the circulating levels of miR-140-5p and miR-142-3p were markedly decreased [55]. Of potential importance, miR-140-5p and miR-142-3p are both highly expressed in blood neutrophils [56]. It is not clear whether increased circulating miR-140-5p and miR-142-3p in obese individuals originated from blood cells, such as neutrophils (potentially reflecting the inflammatory component of obesity), or from a tissue more traditionally associated with obesity.

Conclusions

MicroRNAs, whether considered epigenetic or regulatory, play a major role in human diseases. Aberrant expression of microRNAs has been reported in all cancers. Altered also in metabolic diseases, it is clear that microRNAs and their significance in clinical medicine should be looked into more closely. The ability to detect microRNAs in tissue and biological fluids makes them highly useful for early diagnosis of disease. Perhaps even more significant would be the ability to use microRNAs to treat human disease through the return of normal microRNA levels. It is evident that microRNAs will play a major role in the future diagnosis and treatment of human disease.

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