

MicroRNA in Cancer and Cachexia—A Mini-Review

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MicroRNAs (miRNAs) are short noncoding RNAs with a length of approximately 22 nucleotides that are involved in posttranscriptional regulation of gene expression. miRNAs cover an important role in all biological processes, and aberrant miRNA expression is commonly associated with cancer. Recent discoveries have associated the involvement of miRNA in an increasingly large number of biological processes, including cachexia. The cachexia syndrome is a debilitating state of cancer that is, at least in part, associated with apoptosis. The mechanism through which tumors promote the characteristic distal loss of muscle and fat mass during the cachectic process is still not deeply investigated. In this review, we briefly describe the role of miRNAs in cancer development and cachexia.

Keywords. microRNA; cancer; cachexia.

MicroRNAs (miRNAs) are short noncoding RNAs of approximately 22 nucleotides that are found in all eukaryotic cells and play important roles in almost all biological pathways [1]. In the 1990s, 2 independent studies identified the *lin-4* gene in *Caenorhabditis elegans* as a small noncoding RNA. After that, for a few years, no major discoveries were made concerning miRNA functions [2,3]. Only in 2002 was the first association between miRNA deregulation and cancer made, by studying a frequently deleted chromosomal region in chronic lymphocytic leukemia (CLL; 13q14). This region contains 2 miRNA genes expressed in the same polycistronic RNA, miR-15a and miR-16-1, whose deletion causes the development of the indolent form of CLL [4]. The association between miRNA deregulation and cancer induced many laboratories to focus their research on the study of these small noncoding RNAs in a wide range of cancers, generating large amounts of data supporting the idea that aberrant miRNA expression is the rule rather than the exception in cancer [5]. miRNAs are involved in the control of several cancer-relevant processes, such as

proliferation [6], apoptosis [7], and migration and invasion [8]. Apart from cancer, miRNAs are involved in many other diseases, such as metabolic disorders [9].

Cachexia is a syndrome characterized by a reduction of muscle and fat body masses, and it is often associated with many types of chronic diseases, including cancer [10]. The weight loss during cachexia is not related to starvation but, rather, to tumor presence and the abnormal production of cytokines. In general, patients with lung or pancreas cancers at an advanced stage have asthenia, physical weakness, and difficulty of concentration, symptoms that are often associated with cancer-induced cachexia. The chemical treatment of tumors in patients with cachexia is often characterized by a low dose-limiting toxicity and, unfortunately, by a grade of weight loss that turns out to be positively correlated with patient mortality [11, 12]. Recently, miRNAs have been discovered to play a very important role during the cachectic muscle-wasting process. Many recent studies reported the detection of miRNAs in body fluids such as serum, plasma, milk, urine, and spinal fluid [13–15]. Experimental evidence also describes the presence of miRNAs associated with microvesicles in body fluids. These circulating miRNAs were found in microvesicles with diameters usually ranging from 30 nm to 1 μ m [16]. Although the microvesicle secretion mechanism is still not well understood, there is clear evidence that microvesicles are able to interact with target cells by fusing with the plasma membrane and consequently

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delivering their load [17]. Recent studies demonstrate that cancer-derived microvesicles containing miRNA were able to induce apoptosis of skeletal muscle cells [18].

Role of miRNAs in Cancer

The first evidence of a link between miRNA deregulation and cancer has been described by Calin et al in 2002 [4]. They were the first to associate the frequent deletion of miR-15a/miR-16-1 with CLL, concluding that these 2 miRNAs exert a tumor-suppressor function [4]. This study is considered a forerunner of miRNA research in cancer, motivating, in recent years, many research groups to focus their efforts on studying the causes of aberrant miRNA expression in tumors. Generally, miRNA deregulation in cancer tissues as opposed to normal ones could be related to aberrant epigenetic changes in the methylation status of miRNA promoters [19]. The altered function and/or expression of enzymes implicated in miRNA biogenesis is another mechanism that can cause aberrant expression of miRNAs in cancer. For instance, the expression of Droscha and Dicer, 2 proteins actively involved in miRNA biogenesis, was decreased in 39% of patients with ovarian cancer [20]. Transcription factor activity also plays an important and multifaceted role in the regulative mechanism of miRNA expression [21]. A very important and representative example of cancer-related transcriptional regulation of miRNA expression is the induction of miR-17/miR-92 cluster transcription by the MYC oncogene [22]. miRNAs can also decisively intervene in the modulation of cellular methylation by regulating the expression of the enzymes responsible for epigenetic control. In the past few years, our group identified the miR-29 family as a key regulator for the *ex novo* expression of DNA methyltransferases DNMT3a and DNMT3b in lung cancer [23]. Recently, the transcriptional effect of hepatocyte growth factor receptor c-MET on miRNAs has been investigated. In particular, our laboratory found that c-MET is able, through specific transcriptional factors such as AP1 and ELK-1 under its control, to promote the expression of the miR-221/miR-222 onco-miRNA cluster and to induce a negative feedback with miR-27a. Our data suggest that the effect of c-MET deregulation in cancer is at least in part linked to a deregulated miRNA expression pathway [24, 25]. Finally, because miRNA genes are transcribed by RNA polymerase II, their expression can be also regulated by nuclear receptors [21]. For example an aberrant expression of nuclear receptors such as estrogen receptor α and androgen receptor in cancer is involved in the deregulation of numerous miRNAs [26].

Some miRNAs also play an important role in drug resistance. miRNAs such as miR-19, miR-21, and the miR-221/miR-222 cluster were upregulated several fold in association with this phenomenon [24, 27, 28], while others, such as miR-130a and miR-298, were downregulated, suggesting an important biological role of miRNAs in drug resistance [29, 30]. It is very important to highlight that the role of miRNAs in drug resistance is

tissue specific. For instance, miR-27a is indirectly related to drug susceptibility in ovarian cancer, but it can be directly involved in drug resistance in leukemia [31].

miRNAs have been shown to also have a role as cancer biomarkers in patients. Recently, our group analyzed the miRNA expression profile of 82 patients with colon cancer. Our data demonstrated that a miRNA profile was able to discriminate between colorectal recurrences in lymph nodes and liver, colorectal liver metastasis, and primary hepatic tumor [32].

miRNA and Cachexia

Cachexia is a syndrome consisting in the loss of muscle proteins and fat mass, which has very important clinical consequences in aging, as well as in diseases such as diabetes, chronic heart failure, and cancer [33]. Such syndrome is incurable and occurs in malignancies such as pancreatic and lung cancer. Patients with cachexia cannot be treated with standard therapies because they could easily lead to their death [18]. The loss of skeletal muscle during cachexia is a result of protein degradation subsequent to an altered metabolism in response to tumor progression [34]. Recently, many efforts have been made to better investigate the molecular mechanisms underlying cachexia. *In silico* and *in vivo* approaches found that miR-206 and miR-21 were sufficient and required for atrophy. Their ability to target the transcription factor YY1 and the translational initiator factor eIF4E3 downstream places them as fine regulators of muscle loss and weakness in catabolic conditions [33]. Overexpression of the Pax7 transcription factor has been recently reported in both tumor-bearing mice and patients with pancreatic cancer and weight loss. This abnormal expression of Pax7 induces stem cell differentiation impairment, resulting in muscle atrophy. These data suggest that the muscle microenvironment is able to affect tumor-induced muscle wasting in a consistent way [35]. It has been shown that secreted miRNAs within exosomes can regulate gene expression in recipient cells by targeting their messenger RNAs [17]. More recently, it has been demonstrated that miRNAs can also directly bind proteins, acting as ligands [36]. As early as 2012, our group showed that tumor-secreted miR-21 and miR-29a were able to bind as ligands to human Toll-like receptor 8 (TLR8), corresponding to murine *TRL7*, in immune cells, inducing the TLR-mediated prometastatic inflammatory response [37]. More recently, our group found that muscle cachexia is caused by a high level of shedding from microvesicles containing miR-21 in lung and pancreatic cancer cells. Such microvesicles fuse with myoblasts and cause them to die by apoptosis. This process depends on the expression of TLR7/8 in the myoblasts. The identification of this process may bring us to develop novel approaches to the treatment of cachexia (Figure 1) [18].

DISCUSSION

miRNAs are a class of small noncoding RNAs that play a critical role in the regulation of a large number of biological processes

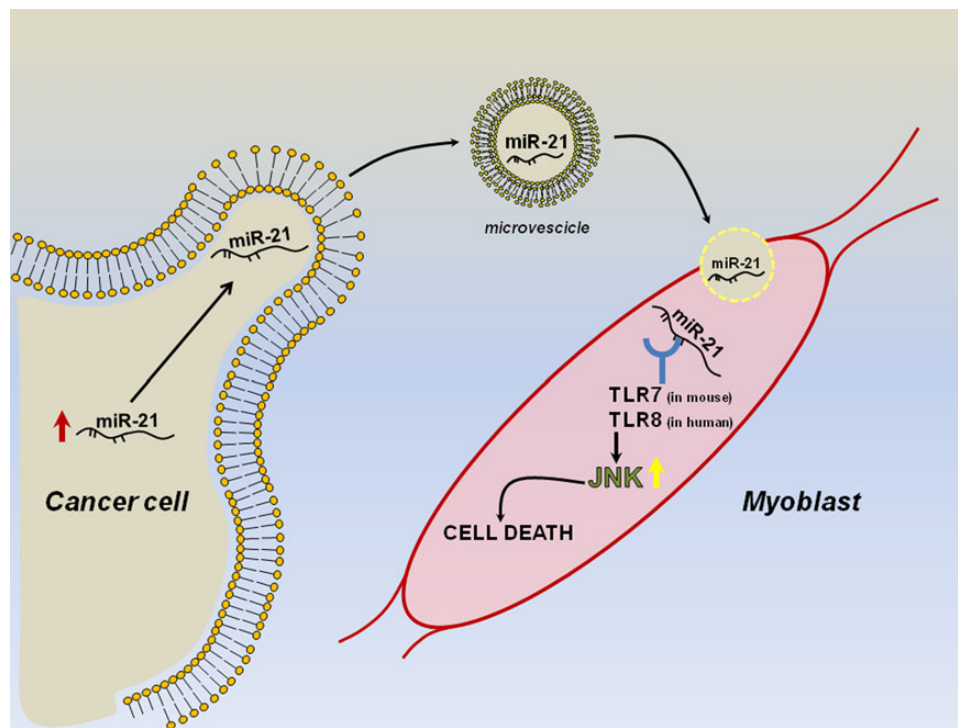


Figure 1. Schematic representation of miR-21 function in cachexia. Abbreviations: TLR7, Toll-like receptor 7; TLR8, Toll-like receptor 8.

and diseases, including cancer [38]. In 2002, our laboratory was the first to associate miRNA deregulation with cancer. This led to a constantly growing number of studies and publications about miRNA expression and cancer. The ubiquitous expression of miRNAs and the pervasive role of certain deregulated miRNAs in cancer processes such as proliferation, apoptosis, and invasion led investigators worldwide to increase their efforts in the understanding of the biology of miRNAs in cancer. In the last 10 years, great progress has been made, but many additional years of study are needed for a complete understanding of the connection between miRNAs and cancer. Recently, new mechanisms of miRNA action have been discovered. For instance, the ability of miRNAs to act as protein ligands or their ability to spread through the body when included within microvesicles yielded new horizons of miRNA research in cancer. It has been recently reported that miRNAs have a role in cachexia, and miR-21 in particular appears to be a key regulator of this process [33]. Our group has recently characterized a new biological mechanism that involves microvesicles and miRNAs: their ability to act as TLR7/8 ligands in humans and mice. This process is very important in tumor-induced cachexia. We discovered that microvesicles were able to shuttle over-expressed miR-21 from cancer cells in tumors to myoblasts, inducing apoptosis. This is a revolutionary mechanism that could enormously contribute to a better understanding of tumor-induced cachexia. We believe that this finding is another

important step toward the comprehension of miRNA function, proving once again the key role that they play in tumors.

Notes

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