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*Review Article*

# The role of microRNAs in gastric cancer

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**Abstract:** Gastric cancer is the fourth most common type of cancer and the second deadliest worldwide; however, the underlying mechanisms of gastric cancer development and progression have not been clearly defined. Recent studies have found that microRNAs (miRNAs), small, non-coding RNA molecules that inhibit translation of mRNAs by binding to their 3'-untranslated region, play a large role in the formation and progression of gastric cancer. There are many families of miRNAs within cells that can be either over- or under-expressed during the development of stomach cancer which target many different mRNA transcripts. These miRNAs are now being studied and explored as potential novel detection and therapeutic strategies for gastric cancer patients. This review will briefly discuss the recent research showing the important roles of microRNAs in gastric cancer.

**Keywords:** Gastric cancer, microRNA, 3'-untranslated region, mRNA transcript

## Introduction

### *Gastric cancer*

Gastric cancer is the fourth most prevalent cancer in the world and is the second most lethal cancer worldwide [1]. Gastric cancers are diseases in which cancerous cells form in the innermost lining of the stomach (mucosa), and are typically adenocarcinomas which are diseases that begin in cells that produce mucus and other fluids [2]. It can be difficult to diagnose stomach cancer until at the advanced stages of the disease are expressed because the symptoms tend to be indistinguishable from other gastrointestinal problems. However, if the cancer or its precursor is identified early, there are several ways for doctors to treat or prevent it. Upper endoscopies, biopsies, computerized tomography (CT), positron emission tomography (PET), and magnetic resonance imaging (MRI) scans, X-Rays, and blood tests are all common diagnostic tests for physicians to use [3-5].

One of the most common causes of stomach cancer is a *Helicobacter pylori* infection [6], which typically stems from a type of inflammation called atrophic gastritis. This bacterium carries out several chemical reactions that convert food to chemicals toxic to the human body, which in turn may cause mutations to the DNA of the stomach cells. Recently, scientists have been working with animal models, which are induced with stomach cancer either chemically or via infection by *Helicobacter pylori* to gain more insight on gastric cancer. It has been found that *H. pylori* are not usually the cause of stomach cancers, but a catalyst in the development of adenocarcinomas, atrophic gastritis, and intestinal metaplasia [7, 8]. This bacterium has also been found to cause several molecular level events in human epithelial cells such as non-specific cellular apoptosis, mutations in gene expression, malfunctions in transduction pathways, and oxidative stress [8]. Their ability to produce these responses in the human body is partly due to their numerous virulence factors that evolve quickly [9]. In a different study, researchers found early inactivation of the *p53* tumor suppressor gene and activation of the *c-met* gene to be indicative of stomach cancer as well [10]. While *H. pylori* is one of the strongest indicators and promoters of stomach cancer, lifestyle habits can be connected to gastric cancers as well.

Stomach cancer can be extremely aggressive and can metastasize quickly. It has been found that pre- and postoperative radiation treat-

ments are beneficial to the patients with gastric cancer. However, these treatments can be harmful to all the other organs that surround the stomach in such close proximity [11]. Several methods for more precise targeting of tumors have been proposed such as 3-dimensional and intensity-modulated radiation therapies, which tend to be more personalized to each patient [12]. While there is a lack of information on the prognostic factors for gastric cancer, as well as a lack of understanding of the different genes that are involved in the gastric tumorigenesis [13], different treatment methods may be recommended depending on the stage of cancer. Physicians are now looking into miRNA functions in gastric cancer as potential diagnostic tools. MicroRNAs have been found to behave in abnormal ways in gastric cancer, sometimes being upregulated and sometimes being downregulated, which can pose more challenge for researchers. This review is intended to give more insight into miRNA functions in gastric cancer and future research that needs to be conducted in this field.

*MicroRNA biogenesis and function*

MicroRNAs (miRNAs) are small (~20-22 nucleotides), single-stranded RNA molecules that do not code for proteins that were discovered in 1993 [14, 15] and have been recently shown to be dysregulated in cancer [16]. They are transcribed from miRNA genes by RNA polymerase II and III to form what are called primary miRNAs, or pri-miRNAs, which an enzyme called Drosha then cleaves to create precursor miRNAs, or pre-miRNAs [17]. This pre-miRNA, which is a hairpin structure, is cleaved once transported into the cytoplasm to create a miRNA duplex, aided by another protein called Dicer. This duplex contains the final, mature miRNA [17, 18]. The duplex will break down and the mature miRNA goes on to dictate cellular events. The less stable strand from the miRNA duplex is typically added to another protein, RISC (miRNA Induced Silencing Complex), whose formation is induced by Dicer, where it can have other effects on the target gene in terms of its protein expression [19]. These effects are most often seen when one strand of the miRNA binds to the 3'-untranslated region (UTR) of the mRNA target sequence [20]. This creation of a double-stranded RNA molecule leads to translational repression.

Short-interfering RNAs (siRNAs) are double-stranded and a perfect match for their mRNA target sequences. In contrast, miRNAs are single-stranded and are an imperfect match to their target sequences, causing bulges in the resulting structure [21]. This implies that miRNAs inhibit translation whereas siRNAs only destabilize the molecule through cleavage. When gene expression profiles are used to compare cancerous and normal tissues, it has been found that miRNAs and also mRNAs are deregulated [22]. This information maybe used to infer that tumorigenesis comes from a change within the miRNome, the collection of miRNAs in the genome, as opposed to a change in a single miRNA that regulates a protein-encoding gene. In addition, it has been found that certain miRNAs are deregulated more often than others, which suggests they play a large role in tumorigenesis [23]. In the beginnings of miRNA research, miRNAs were believed to have similar effects on gene expression (i.e. negative regulation of target mRNA) [24], but research has shown that miRNAs can either repress or activate, depending on the conditions of the cell [25]. It is believed that microRNAs do not function by themselves, but in what are called effector complexes. These are ribonucleoproteins that interact with the miRNA (miRNPs) [26]. The miRNPs are able to gather enzymes and factors that can cleave mRNA and degrade the enzymes that further process mRNA [27]. On the other hand, miRNAs can positively regulate gene expression. This upregulation is specific to the target RNA sequence and associated with the factors gathered by the miRNP [28].

In the past, oncogenes and tumor-suppressor genes were thought of as the main genetic indicators of cancer. Recently, however, miRNAs have been added to that group [29]. When miRNAs are involved in cancer, they are called oncomirs [30]. It has been reported that 50% of genes encoded by miRNAs are located at certain sites called fragile sites where chromosomal rearrangements associated with cancer often occur [31]. Yet, in most cancers, miRNAs are seemingly deregulated. This can be caused by transcriptional deregulation, epigenetic alterations such as DNA methylation, mutation, and DNA copy abnormalities as well as problems in miRNA biogenesis pathways (**Figure 1**). It is assumed that these different mechanisms can either work alone or together in order to

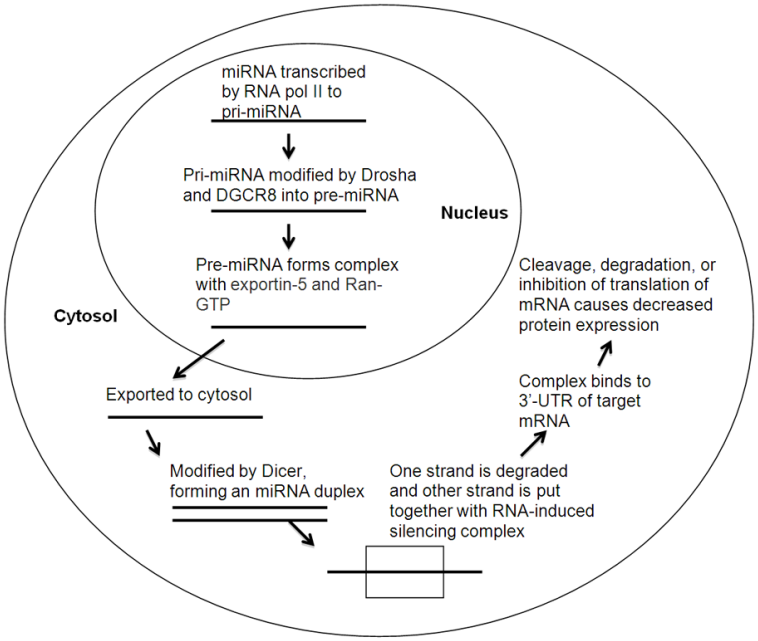


**Figure 1.** Biogenesis of microRNA. This figure demonstrates the synthesis of microRNAs within the cell. MiRNAs are transcribed by RNA polymerase III in the nucleus, starting as pri-miRNAs. This pri-miRNA is then modified by Drosha and DGCR8 proteins into pre-miRNA. This then forms a complex with exportin-5 and RAN-GTP which facilitates exportation into the cytosol. The pre-miRNA is cleaved by Dicer and this forms an miRNA duplex, where one strand of the the miRNA is degraded, and the other, more mature strand is combined with the RNA-induced silencing complex (RISC). This complex can then bind to the 3'-UTR of the target mRNA which leads to degradation, inhibition of translation, or direct cleavage of the mRNA. This will lead to decreased protein expression.

deregulate miRNAs [32]. It has also been hypothesized that miRNAs work in a protein cascade throughout certain cancer-specific protein coding genes. This could potentially change the transcriptional outcome or function of other tumor-suppressor (protein coding) genes. Certain families of miRNAs regulate cell-cycle and cell-cycle exit (senescence) in addition to cell differentiation and proliferation and, if mutated, can cause abnormalities in the cells [33].

In addition to this, if there is a mutation in any given miRNA of a somatic cell, this could lead to tumorigenesis, but if present in germ-line cells, this could be a precursor to cancer [34]. Data suggests that miRNAs are involved in the development of solid tumors as well as aiding their function by controlling these protein-coding genes. Many miRNAs seem to enforce their expression through upregulation across different cancers, suggesting that there are some common mechanisms between these different types of cancers [35], but downregulation of miRNAs also occurs [36].

## MicroRNAs and gastric cancer

### *MiRNAs function as oncogenes and tumor-suppressor genes in gastric cancer*

One of the most notable families of miRNAs in gastric cancer is called *miR-106b-25* [37] that consists of three miRNAs: *miR-25*, *miR-93*, and *miR-106b* [38]. This cluster of miRNAs was found to be the most over-expressed in human gastric cancer cells in their study. This cluster is located on intron 13 of *Mcm17* on chromosome 7. *Mcm17* is important in the transition between the Growth 1 (G1) phase and the Synthesis (S) phase of DNA replication, which allows for the appropriate amount of replication forks to be produced on the DNA [39]. This ensures that the DNA is not replicated more than once. It has been proposed that overexpression of this region causes the miRNA cluster to carry out an oncogenic role [40]. There is a biological tumor suppressor pathway, which involves the transforming growth factor β (TGFβ). Inactivating TGFβ is one of the key steps in the development of tumors [41]. TGFβ effector signals are impaired when *miR-106b-25* is overexpressed in gastric cancer [42]. Gastric tumors that contain elevated levels of *miR-106b-25* precursors show different expressions of each mature miRNA in the family, which implies farther levels of posttranscriptional regulation. This means that numerous changes happen during tumorigenesis besides loss of transcriptional control, which means that the gastric tumors will acquire one, two, or all three of the mature miRNAs [41]. In addition, overexpression of *miR-106b-25* does not only impair the TGFβ signaling, but it also provides an additional mechanism of escape from apoptosis by blocking the translation of a gene called BCL-2-like protein 11 (*BIM*), a proapoptotic gene [43].

Another study focused on the *miR-17-92* cluster, which includes *miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1* and *miR-92*, and together may potentially act as an oncogene [44]. Conversely, *miR-20a* alone has been found to act like a tumor suppressor gene, reducing E2F1 (a type of transcription factor that is important in regulation of the cell cycle in the E2F family) levels [45]. This miRNA binds to the 3'-UTR and regulates translation of certain transcription factors in the E2F family. In cancerous gastric tissue, the levels of both *miR-17-92* and E2F1 are elevated, which indicates a possible negative feedback loop between *miR-17-92* and the E2F family [46].

Certain families of miRNAs can regulate proteins to perform a variety of functions that can alter other cell functions by acting as tumor-suppressor genes in gastric cancer. For example, the *let-7* miRNA family negatively regulates HMGA2, a protein that can control transcription by changing chromatin structure [47, 48]. This protein is thought to be involved in cell proliferation, as it is found in high concentrations in human embryo development, but not in human adults. This is opposite for *let-7*, as it is generally undetectable in the embryonic stages of development, but increases after mature tissues have differentiated [49]. Overexpression of this HMGA2 mRNA, which can be used as a prognostic factor on its own, leads to much higher cell growth which can in turn lead to the formation of tumors. The *let-7* miRNA family negatively regulates this protein because it directly cleaves the mRNA [50, 51]. It has been found that the *let-7* miRNA family tends to act as a tumor suppressor by targeting oncogenes like HMGA2 and RAS [50], as well as their release into the extracellular environment causing a decrease in anti-tumor-forming effects. Because of their role in cell proliferation, it is believed that the *let-7* miRNA family plays an important part in the formation of tumors and metastasis [52]. Despite this, heightened HMGA2 expression in gastric cancer is correlated with higher tumor invasiveness and a poorer prognosis. High HMGA2 expression is a prognostic factor for patients, and the *let-7* miRNA family negatively regulates this protein in gastric cancer [53].

*MiRNAs as modulators of gastric cancer therapeutics*

Using miRNAs as possible therapeutic agents for many types of cancer including gastric cancer has been widely considered among many researchers. One recent study used a plasmid vector with a certain type of miRNA (*miR-516a-3p*) in combination with a delivery reagent called atelocollagen [54]. When this mixture was inoculated into nude mice, the researchers found that this vector allowed for the overexpression of certain proteins made by primary 44As3-tumors, which are common in scirrhous gastric cancer (gastric cancer that involves rapid cancer cell take-over, proliferation, and stromal fibrosis) [55].

In addition to being proficient biomarkers for gastric cancer, a study done by researchers showed that overexpressing miRNAs such as *miR-200c* and downregulating *miR-21* increases chemotherapeutic sensitivity to a cancer drug called Cisplatin [56]. Also, *miR-23a* has been found to lessen the effects of paclitaxel (a cancer drug that inhibits mitosis-induced cell death). This mechanism, however, is incredibly sensitive as miRNA can affect multiple target sequences; further studies are being pursued in order to perfect these protocols [57-59].

In the SGC-7901 cell line, it has been found that miRNAs *15b* and *16* are downregulated, and alterations of their expression have led to changes in response from chemotherapeutic drugs [60]. B-cell lymphoma 2 (BCL2), a protein in the outer membrane of the mitochondria that blocks apoptosis, is directly regulated by *miR-15b* and *miR-16* [61]. This in turn controls whether cells are more susceptible to chemotherapy-induced apoptosis. This is promising for the future of chemotherapeutic drugs in regards to forming multidrug resistance, or MDR. *MiR-15b* and *miR-16*, like all miRNAs, regulate multiple genes and have large impacts on the genome, so this may be a better strategy than developing a drug to target single proteins [62, 63].

Many scientists also suggest that miRNAs which are overexpressed be silenced, while those miRNAs that are under-expressed should be replaced in cancer treatment. For example, a study has found that *miR-100* is a highly variable miRNA being overexpressed or underexpressed depending on the cancer [64]. In gastric cancer, *miR-100* is underexpressed, and its overexpression led to lower amounts of growth in tumors [65]. In addition to the potential methods of therapy mentioned earlier, differentiation therapy has been a promising area of

study. This is a method by which drugs induce cancer cells to differentiate using molecules that are expressed in the affected tissue [66]. This method does not kill all proliferating cells like chemotherapy, but affects solely cancer cells. However, *miR-100* is shown to change the sensitivity of tumors to chemotherapy [67].

Unfortunately, as mentioned earlier, properly diagnosing patients with gastric cancer can be difficult, as there are not many non-invasive diagnostic tests. However, a study was done in order to identify whether the serum miRNAs were different in patients who were already diagnosed with gastric cancer versus healthy patients [68]. Fortunately, it was found that 19 serum miRNAs were significantly upregulated in patients with gastric cancer, but not in healthy patients. Five of these 19 are now used as biomarkers for detection of gastric cancer, and assays using these biomarkers can give telling results about tumor progression in patients with gastric cancer.

While researchers are learning more each day about miRNAs, there is still much to be discovered. A group of scientists found three miRNAs, *miR-451, miR-199a-3p*, and *miR-195*, which may serve as potential markers in gastric cancer, differentiating patients with good versus bad prognoses [69]. Their study indicated that an increased level of *miR-451* correlated with a lower chance of survival. Conflictingly, they mention two other studies that show a decreased level of *miR-451* leading to a worse prognosis in gastric cancer patients. This discrepancy confirms that work still needs to be done in this field of cancer biology. The researchers do state that this could be due to the fact that many miRNAs, such as *miR-451*, have multiple unrelated mechanisms [70, 71].

## The future of miRNAs research in gastric cancer

One of the biggest advantages of using miRNA for therapeutic reasons would be because it can target multiple genes involved in a similar pathway [72]. The researchers argue that by targeting miRNAs that inhibit the normal function of the cell cycle, they are able to knock these proteins out to restore the regular, functioning cell cycle.

In order to make miRNAs more successful in the realm of cancer therapeutics, scientists are discovering ways to modify synthetic miRNAs for easier transfer to host cells *in vivo*. It has been found that miRNAs are prone to nuclease degradation [73] and their processing machinery tends to be insufficient [74] which lowers their bioavailability. By altering certain structural elements such as the 2'-OH ribose or phosphate backbone of synthetic miRNAs, scientists have found that this makes them less likely to succumb to nuclease degradation. After these modifications, the miRNAs can be packaged in viral vectors, nanoparticles, or vectors containing tandem repeats of miRNAs (antisense sponges). These methods of delivery have their downsides as well, including host inflammatory responses, mutations of proto-oncogenes, cytotoxicity, and high cost [75]. In addition, there is a theory that delivering miRNA mimics *in vivo* for therapeutic reasons runs the risk of abnormal accumulation of miRNAs in the cells, which could overwhelm RISC and cause major issues with the functions of normal miRNAs [76]. One of the biggest challenges for delivering miRNAs into tumor tissues is due to the fact that there is inefficient penetration of the miRNA (or miRNA mimic) into the tumor [77] because the tumor's leaky structure leads to inadequate blood perfusion [78]. Another major challenge is that miRNAs are typically unstable and are degraded by nucleases in the blood when inserted into the body [79]. In addition to these challenges, scientists also face the problems of toxicity (as mentioned above), low uptake of miRNAs into cancer tissue [77], and off-target effects of miRNA delivery [80].

Overall, gastric cancer has proven to be a highly skilled and elusive killer, avoiding detection by doctors and in some cases the patients themselves. However, with a lot of the recent information on miRNAs, there is evidence of promise in the future of gastric cancer prevention, prognoses, and therapeutics. There is still much work to be done in this field, but progress is being made daily to understand how miRNAs work and how this can be applied to prevention of gastric cancer.

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## Disclosure of conflict of interest

None.

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