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Chapter

MicroRNA-335-5p and Gastrointestinal Tumors

Pablo M. Santoro, Alejandra Sandoval-Bórquez and Alejandro H. Corvalan

Abstract

Noncoding genomics, i.e., microRNAs and long coding RNAs (lncRNA), is an emerging topic in gastrointestinal tumors. In particular, the coordinate deregulation of miRNA-335-5p across these tumors and its potential clinical applications is an example of this scenario. This chapter discusses the pathogenetic role of miRNA-335-5p in esophageal, gastric, colon, liver, gallbladder, and pancreatic tumors. This pathogenetic role is examined in the context of the competing endogenous network, the language through lncRNA that reduce the quantity of miRNA available to target mRNA. The translational application of miRNA-335-5p, through the aberrant methylation of the promoter region of MEST—its host gene—as a potential biomarker for noninvasive detection of gastric cancer, is also discussed.

Keywords: ncRNA, miRNA-335-5p, gastrointestinal tumors, gastric cancer, competing endogenous, CERNA, DNA methylation, biomarkers

1. Introduction

Gastrointestinal tumors (i.e., esophagus, stomach, colon, liver, gallbladder, and pancreas) are among the most common cancers by incidence and mortality in males and females worldwide [1]. Furthermore, projections of global mortality and disease burden indicate that new cases and deaths from these tumors will increase by 2030 [2]. Given this scenario, understanding of the molecular basis of gastrointestinal tumors is essential to the development of novel strategies for diagnosis and disease treatment. Large genomic studies focusing on protein-coding regions have identified multiple of genes recurrently mutated in gastrointestinal and other human neoplasms [3]. However, molecular classifications based on coding genes do not fully capture the clinical heterogeneity found in gastrointestinal tumors [4]. This observation indicates that other segments of the genome might also contribute to the emerging complexities observed in the development and progression of gastrointestinal neoplasms. In this chapter, we describe recent advances in our understanding of noncoding genome in gastrointestinal cancer. In particular, we will focus on miRNA-335-5p, since not only has it been found to be critically involved in myriad tumors but it has also proved to be a potential biomarker for noninvasive diagnosis of cancer and for the treatment of preneoplastic conditions [5, 6].

2. Noncoding genomics

The traditional view of the unidirectional flow (i.e., DNA-RNA-protein) of genomic data has been reclassified as multidirectional, based on the fact that even though 80% of DNA is transcribed into RNA, only 2% ultimately represent the coding genes which are translated into protein [7]. Therefore, the majority of RNA is defined as noncoding RNA (ncRNA) which in turn includes a wide range of RNA families such as those involved in the translation and splicing of messenger RNA (mRNA) as well as those associated with the modification of ribosomal RNA [7]. ncRNA also plays an essential role in all multiple biological functions, i.e., cell proliferation, apoptosis, cell migration and invasion, and cell differentiation being involved in each of the cancer hallmarks as well [8]. Based on the size of its sequence, ncRNA can be divided into short (~20–200 nucleotides; nt) and long ncRNA (200 to ~100,000 nt) [9].

2.1 Short noncoding RNAs

Short ncRNAs (sncRNAs) are represented by P-element-induced wimpy testis (PIWI)-interacting RNAs (piRNAs), small interfering RNAs (siRNAs), and microRNAs (miRNAs). piRNAs (24–32 nt) are specialized for repression of mobile and other genetic elements in germ line cells (e.g., LINE1 piRNAs and piR-823) [10]. piRNAs and PIWI have been found deregulated in a tissue-specific manner in a variety of neoplasms, opening novel opportunities to diagnosis and treatment of disease [10]. siRNAs regulate posttranscriptional gene silencing and the defense against pathogen nucleic acids (e.g., L1-specific siRNA and oocyte endo-siRNAs) [11]. Therefore, they seem to have great potential in disease treatment, especially as promising epigenetic therapy through the silencing of cancer-related genes [12].

miRNAs represent the largest group of short noncoding RNAs, highly conserved and involved in the posttranscriptional regulation of gene expression in multicellular organisms [13]. miRNAs were discovered in the 1990s while studying fetal development of *Caenorhabditis elegans* [14]. To date, more than 30,000 miRNAs have been found in over 200 species [15]. In humans, the latest miRNA database miRBase release (Release 22.1) contains 2588 annotated mature miRNAs [15]. It is estimated that 60% of coding genes may be regulated by miRNAs. miRNAs have been found deregulated in a tissue-specific manner in human neoplasms, offering novel opportunities for diagnostic assessment and disease treatment [16].

Functional studies have confirmed critical roles of miRNAs in development and disease, particularly in cancer [17]. miRNAs can act as tumor suppressors or oncogenes, and miRNA mimics have shown promise in preclinical and early stages of clinical development [17]. miRNAs reflect the developmental lineage and differentiation state of the tumors being mostly downregulated compared with normal counterpart tissues [18]. Particularly, gastrointestinal tumors cluster together reflecting their common derivation from embryonic endoderm [18]. miRNA-335-5p is among the most frequently deregulated miRNAs in gastrointestinal tumors.

2.2 miRNA-335-5p structure and regulation of its expression

Although initially described in developmental biology, as differentially expressed and maternally imprinted during mouse and human lung development [19], later studies have shown that miRNA-335-5p is extensively deregulated in human tumors [20]. miRNA-335-5p is a transcript located on chromosome 7q32.2, in the second intron of the mesoderm-specific transcript homolog (MEST)

gene [21], which encodes 17 different mRNAs [22]. In humans, the mature sequence of miRNA-335-5p forward strand, miRNA-335-5p, corresponds to 16 -UCAAGAGCAAUAACGAAAAAUGU-38 (http://www.mirbase.org, accession: MIMAT0000765, ID: hsa-miR-335-5p) [23]. The strong correlation between the expression of miRNA-335-5p and its host gene MEST suggests that the mechanism responsible for silencing miRNA-335-5p expression should be the promoter methylation of MEST [5, 24, 25] (**Figure 1**). The promoter of MEST gene contains three CpG islands upstream of transcriptional start site [26]. The treatment with 5-aza-2′-deoxycytidine (5-aza-dCyd) restores the expression levels of miRNA-335-5p in hepatocellular and gastric cancer [24, 27]. Furthermore, an inverse correlation between expression levels of miRNA-335-5p and its methylation status was revealed in these cancer tissues [24, 27].

2.3 miRNA-335-5p and gastrointestinal malignancies

The coordinated deregulation of miRNA-335-5p across the gastrointestinal tract neoplasms implied the relevant role of this microRNA in these tumors. Esophageal adenocarcinoma (EAC) is one of the fastest rising incidences of cancers with a dismal prognosis [28]. EAC is the final stage of Barrett esophagus (BE), an adaptive response to chronic gastroesophageal reflux in which the squamous epithelium of the esophagus is replaced by glandular columnar epithelium [28]. By combining multidimensional genomic measurements from the TCGA, Xi and Zhang [29] proposed a genomic signature of five differentially expressed miRNAs including miRNA-335-5p that can be applied for novel diagnostic approaches and disease treatment. Gastric cancer (GC) remains the fifth cause in cancer incidence and the third cause of death by neoplasms worldwide [1]. GC is a highly heterogeneous disease with unique ethnogeographical associations [30–32]. Profiling studies have not identified miRNA-335-5p as part of any miRNA signatures with clinical significance. However, gene-specific approaches suggest that miRNA-335-5p is downregulated in GC and clinically associated with advanced TNM stage and worse prognosis [5, 33]. Functional studies have shown that exogenous expression of miRNA-335-5p deregulated many biological cell processes such as cell cycle, proliferation, apoptosis, migration, invasion, and metastasis [5, 25, 33, 34] (**Figure 2**).

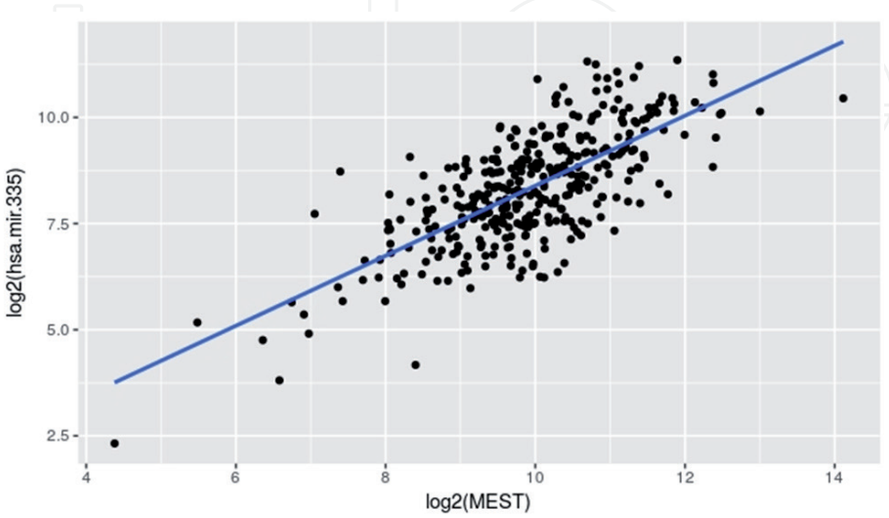


Figure 1.
Linear regression model (blue line) using RNAseq data from 368 tumor samples from the stomach adenocarcinoma from The Cancer Genome Atlas (TCGA) consortium (taken from Sandoval-Bórquez et al. [5] with permission).

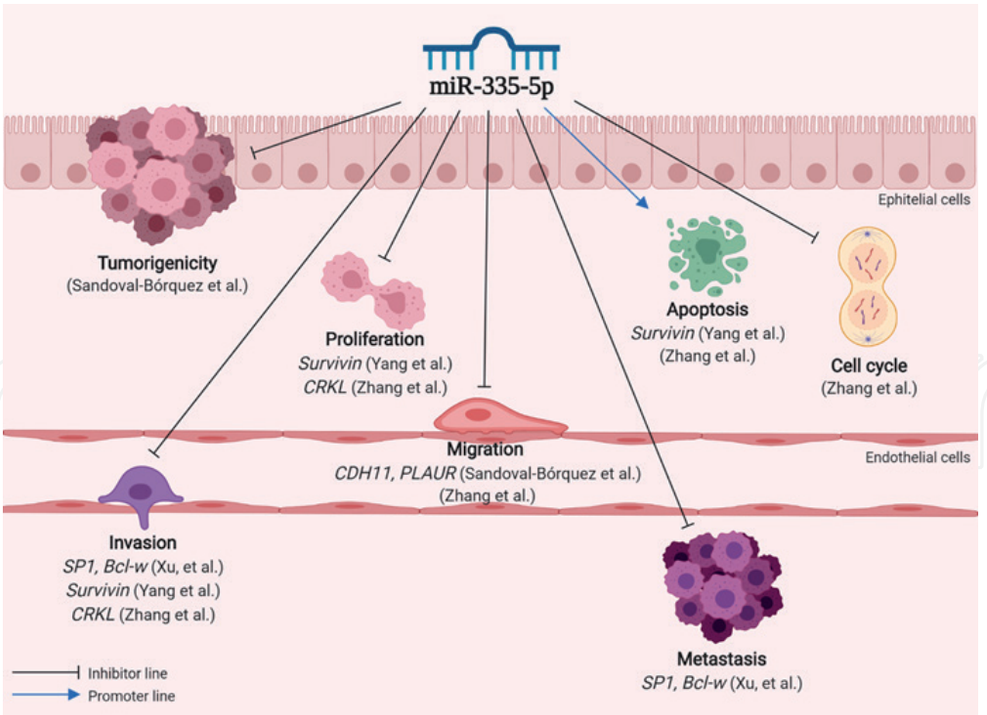


Figure 2.
Cellular processes by which miRNA-335-5p contributes to their regulation though of different target genes in gastric cancer cell lines. miRNA-335-5p inhibits tumorigenicity, cell cycle, proliferation, apoptosis, migration, invasion, and metastasis [5, 25, 33, 34]. Abbreviations: CRKL, V-crk avian sarcoma virus CT10 oncogene homolog-like; CDH11, cadherin 11; PLAUR, plasminogen activator urokinase receptor; SP1, specificity protein 1; BCL-w, BCL2-like 2 (taken from Sandoval-Bórquez et al. [5] with permission).

Of note, upregulation of miRNA-335-5p might be associated with tumor recurrence, the major factor of treatment failure in this disease [35].

Colorectal carcinoma (CRC) is the first cause of death by cancer in developed countries [1]. In this tumor downregulation of miRNA-335-5p has been associated with microsatellite instability (MSI) [36] and ability to discriminate between non-serrated and serrated adenomas [37]. Even though it has been described as upregulated in tumor samples relative to normal mucosa, functional studies have shown that its overexpression inhibits invasion and metastasis in CRC cell lines [38, 39]. The number of deaths for hepatocellular carcinoma (HCC) is nearly equal to the worldwide annual incidence of newly diagnosed cases [40]. Almost 80% of HCC is attributed to chronic hepatitides B and C which evolve to cirrhotic/fibrotic liver and ultimately HCC [41]. Screening of multiple miRNAs identified miRNA-335-5p among unique seven miRNA signatures that could be associated with the development HBV-related HCC [42]. Gallbladder carcinoma (GBC) is an infrequent but highly lethal biliary tract tumor mostly associated with the presence of gallstones and chronic inflammation [43]. Tumor suppressor and cancer-prone miRNAs have been identified in GBC including the downregulation of miRNA-335-5p, which together with other microRNAs produces a signature clinically associated with prognosis and prediction of treatment response [44]. Gene-specific analysis in paired tumor and normal tissues also confirms downregulation of miRNA-335-5p in association with histologic grade, stage, presence of metastases, and poor survival [45].

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal malignancy, and because of the late presentation, as few as 20% of patients are candidates for curative treatment. Ectopic expression of miRNA-335-5p in pancreatic cancer cell lines significantly suppressed cell growth by inhibiting c-Met [46]. Other malignancies in which miRNA-335-5p is deregulated have been comprehensively described Luo et al. [20].

2.4 Long noncoding RNAs and the competing endogenous network of miRNA-335-5p in gastrointestinal malignancies

Long ncRNAs, which span from 200 to ~100,000 nt, make up the largest portion of the human noncoding transcriptome [47]. lncRNAs are essentially regulatory molecules implicated in multiple cellular processes in a tissue-specific manner [48]. Multiple reports have compiled the emerging role of the deregulated expression of lncRNAs in human tumors, and based on tissue-specific transcription, this novel class of genes holds strong potential as biomarkers and new therapeutic opportunities in cancer [49–52]. lncRNAs reduce the quantity of miRNA available to target mRNA (i.e., "sponges"), through the miRNA response elements (MREs) contained within the lncRNA and the 3′UTR of mRNA, which serve as miRNA-binding sites [53]. Based on the relevance of these interactions, a new language has been proposed—the competing endogenous RNA (CERNA)- [52]. In the case of miRNA-335-5p, few CERNA networks have been described, which indicate that this is an emerging area of research in gastrointestinal tumors. As shown in **Table 1**, the lncRNA nuclear-enriched abundant transcript 1 (NEAT1) facilitates Sora resistance in HCC cells via negative regulation of miRNA-335/c-Met/Akt pathway that suppresses apoptosis [54]. The misato family member 2 (MSTO2P) has shown a high expression in parallel to a significantly low expression of miRNA-335-5p in clinical samples as well as in vitro [55]. As of this publication, no mRNA targets for this network have been described. Nuclear-enriched abundant transcript 1 (NEAT) is also upregulated in GC and promotes proliferation, migration, and invasion via targeting the miRNA-335-5p/ROCK1 axis [56]. The zinc finger E-box binding homeobox 1 antisense RNA 1 (ZEB1-AS1) has shown to be critical for the proliferation and invasion of GC cells by regulating miRNA-335-5p [57]. However, the mRNAs associated with this CERNA network are currently unknown.

Further characterization of CERNA networks associated with the deregulation of miRNA-335-5p could be taken from the comprehensive evaluation of genes involved in metastasis and tumor invasion pathways after exogenous miRNA-335-5p expression [5]. Through this experiment up to 19 out of 62 (30.6%) genes were significantly increased [5]. Of note, miRNA-335-5p can target several messenger RNAs, and deregulation of miRNA-335-5p can effectively affect multiple signaling pathways leading to metastasis and tumor invasion [58]. In this scenario, ingenuity pathway analysis (IPA) narrowed the field to nine upregulated genes (CDH11, COL4A2, CTGF, CTSK, MMP7, PDGFA, PLAUR, TIMP1, and TIMP2) (**Figure 3**). Most of these upregulated genes belong to intracellular signaling pathways in cancer such as PI3K-Akt (COL4A2, MYC, PDGFA, SPP1), proteoglycans (HIF1A, MYC, PLAUR, TGFB1), and Hippo (CTGF, MYC, NF2, TGFB1). Among these genes, PLAUR significantly increased mRNA levels after knockdown of miRNA-335-5p expression in GC cells. PLAUR is a membrane-bound glycoprotein with a GPI anchor that encodes the receptor of urokinase-type plasminogen activator and binds and activates PLAU [59]. This activated serine protease converts plasminogen to plasmin, degrading all components of the extracellular matrix and promoting invasion and metastasis [60]. Furthermore, PLAUR has signaling properties through interactions with membrane-bound integrins, which are able to affect migration and cell proliferation [61]. In GC, the overexpression of PLAUR has been reported to be closely related to cell invasion and metastasis [62, 63]. In vitro analysis also showed a significant increase of PLAUR expression in miRNA-335-5p knockdown cells, and, consequently, cells overexpressing miRNA-335-5p exhibited a low level of PLAUR. Accordingly, elevated levels of PLAUR were observed in tumor tissues when compared with their paired non-tumor mucosa [5]. Another relevant gene overexpressed after exogenous miRNA-335-5p expression was

lncRNA name	ID	Abbreviation	Target coding gene	Topic	Validation	Reference
Nuclear-enriched abundant transcript 1	ENSG00000245532	NEAT1	c-Met	Hepatocellular carcinoma	In vitro/in vivo	Chen and Xia [54]
Misato family member 2, pseudogene	ENSG00000203761	MSTO2P	Unknown	Gastric cancer/metastasis	Clinical/in vitro	Li et al. [55]
Nuclear-enriched abundant transcript 1	ENSG00000245532	NEAT1	ROCK1	Gastric cancer	Clinical/in vitro	Wang et al. [56]
ZEB1 antisense RNA 1	ENSG00000237036	ZEB1-AS1	Unknown	Gastric cancer	Clinical/in vitro/in vivo	Zhang et al. [3, 57]

Table 1.
Competing endogenous RNA (CERNA) associated to miR-335-5p.

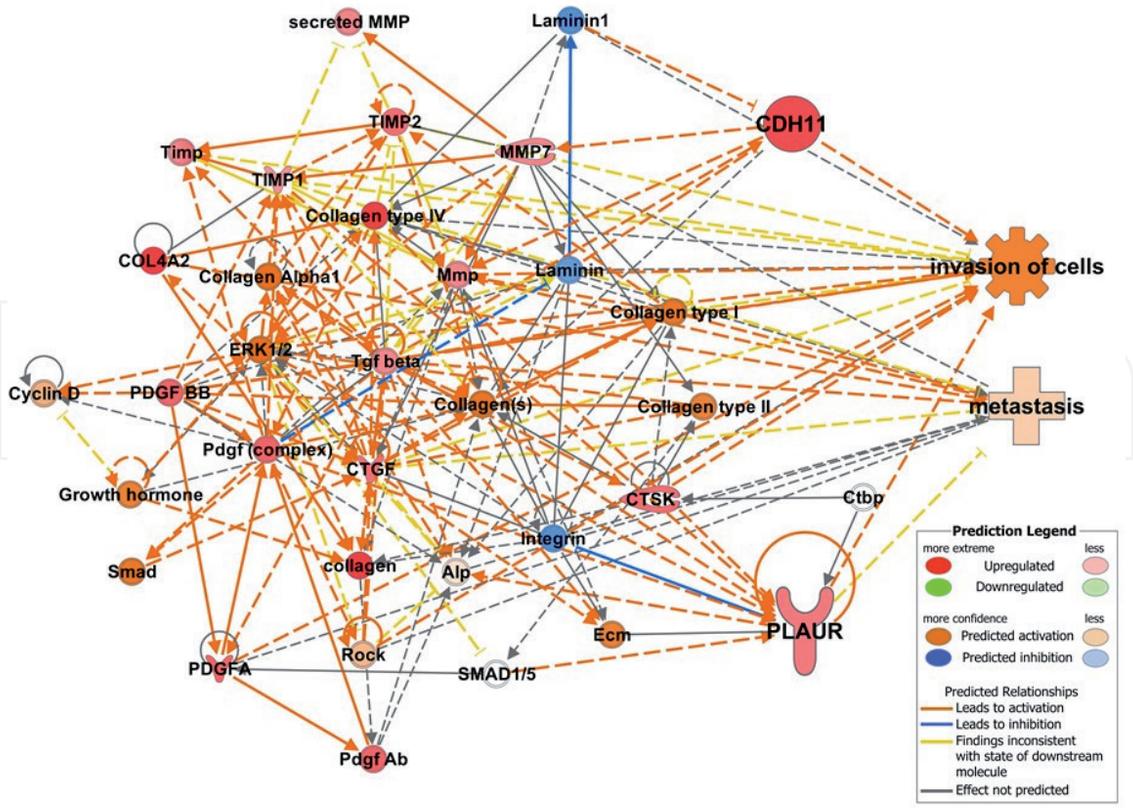


Figure 3.
*Ingenuity pathway analysis (IPA) for network enrichment analysis identified metastasis and invasion downstream genes of miRNA-335. A network of nine significantly overexpressed (red) genes during miRNA-335 inhibition. The MAP tool shows activation and inhibition of neighboring genes and predicts activation of metastasis and invasion of cells in silico. *p < 0.05 (taken from Sandoval-Bórquez et al. [5] with permission).*

CDH11, which encodes a type II classical cadherin, an integral membrane protein that mediates calcium-dependent cell-cell adhesion [64]. CDH11 has been reported as deregulated in various tumor types, suggesting a role in metastasis and tumor invasion [65, 66], and its overexpression was found in advanced cases of gastric cancer [67]. Binding experiments demonstrate the presence of direct targeting of miRNA-335-5p in the CDH11 gene [5]. Interestingly, preliminary data of potential targets of miRNA-335-5p in association with PLAUR and/or CDH11 reveal 15,898 new lncRNAs or 27,688 transcripts for further exploration of the role of CERNA in metastasis and tumor invasion pathways.

2.5 Translational applications of miRNA-335-5p

Methylated cell-free DNA in plasma has emerged as a potential biomarker for diagnosis, prognosis, and prediction of treatment response in gastrointestinal tumors [24, 68]. In addition, several studies have shown that downregulation of miRNAs is associated with DNA methylation of the promoter region of its host genes [69].

Envisaging the clinical application of the downregulation of miRNA-335-5p in GC, Sandoval-Bórquez et al., [5] expand to plasma the reported inverse correlation between the expression of miRNA-335-5p and aberrant promoter methylation of its host gene (MEST) in tissues and cell lines [24] (**Figure 4A**). Furthermore, these authors demonstrated that this aberrant hypermethylation could be a surrogate biomarker for noninvasive diagnosis of GC since it was significantly found in GC cases in comparison with healthy donors ($p = 0.029$, Pearson's correlation) (**Figure 4B**).

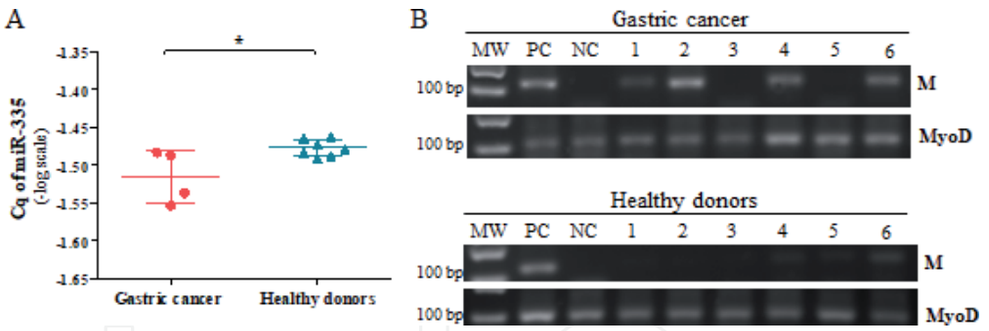


Figure 4.
Potential clinical application as noninvasive detection of GC by the downregulation of miRNA-335-5p. In (A) expression of miRNA-335-5p in plasma from GC patients and healthy donors by Cq of miRNA-335-5p, data were transformed to logarithmic values (−log), and results indicate the mean ± SD. In (B) methylation-specific PCR of the promoter region of MEST gene in plasma from GC patients and healthy donors, MyoD was used as a control of DNA conversion. MW, weight marker; M, PCR product with primers specific for methylated promoter region of MEST, host gene of miRNA-335-5p; PC, positive control of methylation (methylated GC cell line); NC, negative control of methylation (peripheral blood lymphocytes) (taken from Sandoval-Bórquez et al. [5] with permission).

3. Final conclusions

In this review, we summarize the role of miRNA-335-5p in gastrointestinal tumors with a focus on GC. We also explored the role of miRNA-335-5p in noncoding and coding gene networks and its downstream signaling pathways involved in the biological effects of tumor growth, invasion, and metastasis. This evidence supports the potential use of miRNA-335-5p in noninvasive diagnosis as well as therapeutic prospects in gastrointestinal cancers.

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Conflict of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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