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Review

Gastric Juice MicroRNAs as Potential Biomarkers for Screening Gastric Cancer: A Systematic Review

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Abstract. *Background/Aim: To date, the combination of gastroscopy with biopsy remains the only test validated for screening gastric cancer (GC). Currently, analysis of circulating microRNAs (miRNAs or miRs) is providing interesting information on GC prognosis, but since these molecules are shared by several types of cancer, its clinical use could be questionable and difficult. MicroRNAs in gastric juice (GJ) could represent a cogent alternative to screening GC by biopsy. Materials and Methods: We investigated the pertinent literature dealing with GC GJ microRNAs through four popular search engines (PubMed, Science Direct, Scopus and Google Scholar). Results: As of 2017, only four studies had been published and were all from Chinese experience. MiR-421, miR-129, miR-21, miR-106a and miR-133a were the five molecules studied in the GJ of the enrolled patients. Conclusion: The GJ miRNA test is reliable and reproducible. The discussed GJ miRNAs appear to be new potential biomarkers for the screening of GC.*

To date, gastric cancer (GC) remains one of the most frequent and lethal digestive malignancies worldwide (1). Differently from advanced GC (AGC), early GC (EGC) has an excellent prognosis with a 5-year survival rate higher than 90%; however, differently from Japan and Korea where GC screening is commonly conducted, EGC is rarely detected through screening in USA and Europe (2, 3). Since non-endoscopic screening tests for GC, such as the determination of tumor markers in serum or gastric juice (such as carcinoembryonic antigen (CEA), carbohydrate antigen 19.9 (CA19.9) and cancer antigen 72.4 (CA72.4) have not achieved satisfactory sensitivity and specificity, recently medical studies have drawn attention to another class of molecules, the so-called microRNAs (miRNAs or miRs) (4-9). These small RNA segments, although coding for no protein, regulate expression of several genes at the post-transcriptional level and act as potential oncogenes (oncogenic miRs or oncomiRs) and antioncogenes (tumor-suppressor miRs) (10). When obtained from serum or tissue (mucosa) of patients with GC, a number of studies have demonstrated that several microRNAs can be used as promising markers for prognosis of these patients (11-13). Some circulating molecules have been also examined as potential heralds of gastric malignancy, but results were conflicting, impairing their adoption in clinical practice (14-16). In this review study, our aim was to define whether analysis of miRNAs isolated from gastric juice (GJ) of patients with GC patients could parallel or surpass the results from the examination of plasma or tissue molecules and thereby be considered a potential biomarker tool for screening GC.

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Materials and Methods

We examined the English-language literature existing as of 2017 dealing with GJ miRNAs obtained from GC patients. The investigation was conducted through four popular search engines (PubMed, Science Direct, Scopus and Google Scholar). Gastric cancer, gastric juice, microRNA, miRNA and miR were the key words utilized for searching. Articles which did not include analysis of intragastric miRNAs were excluded from this review.

Results

We found only four studies dealing with GC miRs in GJ (6-9). All of them came from China: three were from the city of Ningbo (6-8) and one from Yangzhou (9). *miR-421, miR-129, miR-21, miR-106a* and *miR-133a* were the five microRNAs studied. Main features of the studies are summarized in Table I.

The first study by Zhang *et al*. was on *miR-421*. *miR-421* was the first analyte examined by the study group from Ningbo (6). After collecting GJ from 141 individuals, of which 42 had GC, (seven early GCs and 35 advanced GCs), RNA was extracted, processed to reverse transcription (RT) using the miScript RT kit, purified, cloned and sequenced; finally, to verify the stability and reproducibility of this miR, the test was repeated on stored GJ specimens in two independent experiments (14). The authors demonstrated that the GJ levels of *miR-421* in patients with GC were significantly lower than those with benign gastric diseases ($p<0.001$). Additionally, for the detection of early GC, the combined use of *miR-421* and CEA in GJ showed a remarkable improvement compared with the adoption of serum CEA alone ($p=0.029$). As for *miR-421* obtained from endoscopic mucosal biopsies, they found that all GC specimens showed lower expression compared with those from adjacent normal specimens ($p=0.001$).

miR-129 in GJ was the second molecule studied in Ningbo (7). The procedure was the same as that adopted for GJ *miR-421*. In this study, 42 patients with GC had significantly lower levels of miR-129-1-3p ($p=0.007$), *miR-129*-2-3p ($p=0.003$) and the combination of two ($p=0.003$) in GJ compared with 99 individuals with benign gastric diseases. Additionally, the GJ level of *miR-129*-1-3p was significantly lower in male patients with GC than in female ones ($p=0.043$).

In their subsequent work, the group from Ningbo discovered that both *miR-21* and *miR-106a* were less expressed in GJ of 42 patients with GC than in 99 patients with benign gastric diseases ($p<0.001$ for both molecules) (8). Moreover, both GJ microRNAs were associated significantly with GC stage ($p<0.001$ for both analytes) and the level of *miR-21* in the intestinal GC type was higher than in the diffuse ($p=0.003$) or mixed ($p<0.001$) histological types. Finally, when used as biomarkers, the combined use of *miR-21* and *miR-106a* in GJ with serum CEA measurement revealed a notable improvement for the detection of early GC ($p<0.05$) compared to absence of significance for tests alone.

miR-133a was investigated by a group from Yangzhou (9). The miScript analytic kit was the same as that of the group from Ningbo. The Authors discovered that *miR-133a* exhibited lower expression in tissues ($p<0.001$) and GJ ($p<0.001$) of 62 patients with GC than those with other benign diseases and healthy controls (142 individuals).

Discussion

Although circulating CEA, CA19.9 and CA72.4 have a notable prognostic utility for predicting recurrence and metastasis of GC following surgery and chemotherapy, as of 2017, none of these tumor antigens has offered significant value for the screening of GC; as a consequence, the combination of gastroscopy with endoscopic biopsies still represents the only valuable current method for GC diagnosis and screening (4, 5). Through the last decades the search for the ideal GC marker has gone on: while many researchers continued investigating blood, some authors started analyzing another biological material, the GJ (17). According to them, GJ could represent an excellent source of GC biomarkers because these are directly released by the tumor without being eliminated by the liver (17). Over time, analysis of intragastric fluid of patients with GC has provided interesting results in terms of classification, staging and prognosis of this cancer (3, 18-21); however, research on conventional tumor antigens (such as CEA, CA19.9 and CA72.4) present in this organic fluid has not met the high expectations (4).

Consequently, the scientific community has directed its attention to another class of promising molecules: microRNAs (12). These are small non-coding RNAs which regulate numerous genes in human tumorigenesis including GC; given their important role, since their first description in 1993, research on miRNAs has piqued ongoing interest worldwide (11, 12). For GC, from 1993 through 2011, such molecules have been investigated only in tissue and serum specimens of patients with GC: these analyses provided important information on prognosis and prediction of the patients but for efficient screening, even in this case, they yielded conflicting results thereby limiting their clinical application (14-16). In fact, all the microRNAs tested in peripheral blood were shared by several types of cancer (such as colorectal, ovarian, breast, prostate and bladder cancer) making the assessment of tumoral paternity difficult (9).

In 2012, the first analysis of an miRNA in GJ came to light: the study group from Ningbo demonstrated that compared with patients having benign gastric diseases, patients with GC showed significantly lower levels of *miR-421* in GJ ($p<0.001$). Considering that *miR-421* is an established oncomiR involved in the up-regulation of tumor-associated nuclear receptors, as well as the significantly higher sensitivity and specificity of its measurement in GJ compared with that in serum, the authors assessed this microRNA as a potential new biomarker for screening GC (6). They also demonstrated the feasibility and reproducibility of such a test: after RNA extraction, in fact, *miR-421* appeared to be highly stable in GJ and easily examinable through a conventional real-time quantitative reverse transcription-polymerase chain reaction (RT-qPCR) followed by purification, cloning and sequencing (6).

Table I. *Main features of the microRNAs previously studied in gastric juice (GJ).*

Reference no.	GJ miR no.	Role	GC/non-GC* patients	EGC/AGC	Mean age of patients with GC (years)	miR assay kit	GJ expression level (GC *vs*. non-GC group)
6	421	Oncogene	42/99	7/35	64.2±13.2	miScript RT kit	Low *vs*. high ($p<0.001$)
7	129	Anti-oncogene$^+$	42/99	7/35	64.2±13.2	miScript RT kit	Low *vs*. high ($p\leq0.007$)
8	21	Oncogene	42/99	7/35	64.2±13.2	miScript RT kit	Low *vs*. high ($p<0.001$)
8	106a	Oncogene	42/99	7/35	64.2±13.2	miScript RT kit	Low *vs*. high ($p<0.001$)
9	133a	Anti-oncogene$^+$	62/142	n.a/n.a.	61.5±12.7	MicroRNA reverse transcription kit	Low *vs*. high ($p<0.001$)

GC: Gastric cancer; EGC: early GC; AGC: advanced GC; GJ: gastric juice; n.a.: not assessed. *Individuals with gastric ulcer, atrophic gastritis, minimal gastritis or normal mucosa at gastroscopic biopsy. $^+$also known as tumor-suppressor gene.

miR-129 acts as a tumor suppressor by regulating G_1/S phase transition and apoptosis (7). Former studies showed that *miR-129* levels in gastric, colorectal and lung adenocarcinoma tissues were significantly lower than in their corresponding normal tissues; in the reported work, GJ levels of this miR were significantly lower in patients with GC than in non-oncological patients (7, 22, 23). Hence, differently from its serum counterpart, this miR, because of its exclusively intragastric presence, could also be considered a new choice for the diagnosis of GC (7). Similarly to *miR-129*, GJ levels of *miR-21* and *miR-106a* were also lower in patients with GC compared with non-oncological patients; moreover, both miRs were up-regulated in plasma and tissue of patients with GC and down-regulated in patients with benign gastric diseases (8). Of interest, in this work, Cui *et al*. for the first time offered one possible explanation of the phenomenon causing lower and higher levels of miRs in GJ and tissue/serum, respectively, of patients with GC (8). According to them, in order to maintain and promote oncogenesis, GC was able to reduce the export of oncogenic miRs (such as *miR-21* and *miR-106a*) into GJ and enhance, at the same time, their release into the extracellular environment; here, they might participate in a crosstalk process which could support the neoplastic growth profile (8).

miR-133a is a muscle-specific microRNA, so-called myomiR, which promotes muscle growth, regulating earliest differentiation of myogenic stem cells into myoblasts (24). Regarding GC carcinogenesis, it acts as a tumor-suppressor gene: the restoration of its lower expression (as occurs in GC), in fact, can inhibit cancer cell proliferation, invasion and migration (9). Shao *et al*. found that *miR-133a* exhibited significantly lower expression in the GJ of 62 patients with GC ($p<0.001$) than in those with other benign diseases and healthy controls (142 individuals): their conclusion was that down-regulation of miR in GJ was a reliable clinicopathological biomarker for GC screening (9).

Despite such promising results, it appears evident that further studies are needed to consolidate the role of these and other microRNAs for the screening of GC. Furthermore, for the future, such molecules in GJ could be investigated for development of targeted therapies.

Conclusion

Differently from conventional tumor antigens (such as CEA, CA19.9 and CA72.4) in serum and GJ and diverging from plasma or tissue miRs, miRs in GJ seem to be statistically significant biomarkers for the screening of GC (6-9, 17). miRs appear to be stable in GJ: this feature makes the procedure of extraction and amplification feasible and reliable. More studies on these and further molecules are needed in order to improve the diagnostic strength of this such a new test.

Conflicts of Interest

The Authors declare no conflicts of interest in regard to this study.

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