Role of microRNAs in the progression and metastasis of gastric cancer

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ABSTRACT

Despite the discovery of cancer biomarkers and advancement in endoscopy, gastric cancer (GC) ranks third among the cancer death worldwide due to lack of early detection and poor understanding of its progression at the molecular level. Hence, early detection of GC is highly crucial for improving prognosis. MicroRNA's (miRNA's) belong to a group of non-coding RNA which are ~18–25 nucleotides long and could regulate the target gene expression at posttranscriptional level. Many findings have reported that dysfunction of miRNA could modulate the key biological processes and that has been associated with number of inflammatory disease including GC. As miRNA expression was associated with GC progression such as proliferation, migration, invasion, and apoptosis, the detection of miRNA in blood, gastric juice, tissues, and urine could offer new potential biomarkers for GC and design the novel therapeutic targets for GC. This review focuses on the biogenesis, role of miRNA in GC progression and metastasis, recent advancement and challenges in using miRNA as potential biomarkers for GC. Thus the information on miRNA's can serve as potential targets in early detection of GC to improve the survival rate and in the development of therapeutic targets.

1. INTRODUCTION

Gastric cancer (GC) ranks fourth among the most common cancer and ranks third in the cancer death worldwide. There were 19,293,789 cases for cancer reported in 2020, of which nearly 5.6% i.e. 1,089,103 cases were of stomach cancer. The total number of death's in 2020 was about 9,958,133 out of which 768,793, that is, 7.7% death cases were of GC. The incidences have increased by 1.3% and mortality rate has also increased by 0.9% across the world [1]. GC is a multifactorial disease [2]. Genetic factors along with environmental condition play an important role in the cause of GC. Lifestyle changes that increase the vulnerability to GC includes smoking, high intake of salty, smoke foods, low dietary fiber, obesity, and radiation [3]. Besides, the above factors, *Helicobacter pylori* colonization in stomach lining is found to be the main etiological factor for GC. The gastric pathogen has the capacity of forming colonies in the mucosal membrane and evokes an immune response. This results in the generation of reactive oxygen species due to the infection which could damage the DNA. Men have two-fold higher risks than the women of developing GC. This may be due to estrogens which protect a person from developing GC. Furthermore,

consumption of anti-estrogen medicines like tamoxifen might increase the incidence of a person getting GC [4]. Peritoneal metastasis (PM) occurs in around 53 to 66% of patients who has clear cut metastatic GC. CT or computed tomography scan was used most commonly as the non- invasive method to diagnose PM. Early diagnosis and detection of the GC were quite important as there would be minimal treatment required and the person could prevent unnecessary surgical treatment. However, CT detection have low sensitivity and high specificity as the clinical signs such as omental cake, parietal peritoneum thickening, and deposition of large number of ascites would appear in the later stage of cancer. Even when the patient undergoes multiple clinical tests, on an average 16.7% of peritoneal metastatic cancer are not detected. Laparoscopy should be done to patients who are potentially resectable advanced GC to detect occult PM. For the past few decades, microRNA (miRNA) has emerged in the field of cancer as biomarkers. miRNA's, a class of non-coding RNA(~22 nucleotide long) plays a very important role in the regulation of gene expression [5,6]. miR-34a will increase the expression of genes which are related to apoptosis, miRNA-373, miRNA-10b, miRNA-520c will encourage tumor metastasis and invasion [7]. Nearly 140 miRNA were found to be up regulated (miRNA-9, miRNA-21, miRNA-17-92, miRNA-145, miRNA-224, etc.); 17 miRNA were downregulated (miRNA-449a, miRNA-223, miRNA-421, and miRNA-34) and these miRNA play an important role in GC disease diagnosis and serve as a biomarker for the early detection of GC and potential therapeutic targets [8].

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2. miRNA BIOGENESIS AND MECHANISM

Various types of ribonucleic acid such as messenger RNA, miRNA, transfer RNA, and ribosomal RNA are found in eukaryotes. Besides the role of RNA in protein synthesis, miRNA's belongs to non-coding RNA family, are involved in regulation of gene expression at the post-translational level. The biogenesis will start either co-transcriptional or post-transcription by processing RNA polymerase. The biogenesis can occur either by canonical or non-canonical pathway which are as follows [8,9].

2.1. Canonical Pathway

miRNA genes were transcribed by RNA polymerase II into initial transcript called pri-miRNA, which is about ~500 to 3000 bp long [8]. These pri-miRNA's were further modified/transformed to hairpin shaped precursor miRNA called pre-miRNA's (~60–70 nucleotide long) with the help of DROSHA or DGCR 8 enzyme. At this stage, the precision processing was very crucial for pre-miRNA as this would determine the target sequence in mRNA for gene silencing. The pre-miRNA was taken into the cytoplasm by exportin-5 and later on dicer (belongs to RNase III) cleaves the pre-miRNA and give rise to ~20–24 nucleotide RNA duplex, where one strand was loaded into RNAinduced silencing complex which has Argonaute [Figure 1]. This complex would muzzle/silence the target miRNA and could regulate the gene expression at post-translational level. Thus, these miRNA's could play a key role in the animals including roles in cell apoptosis, grow, and cell proliferation [8-11].
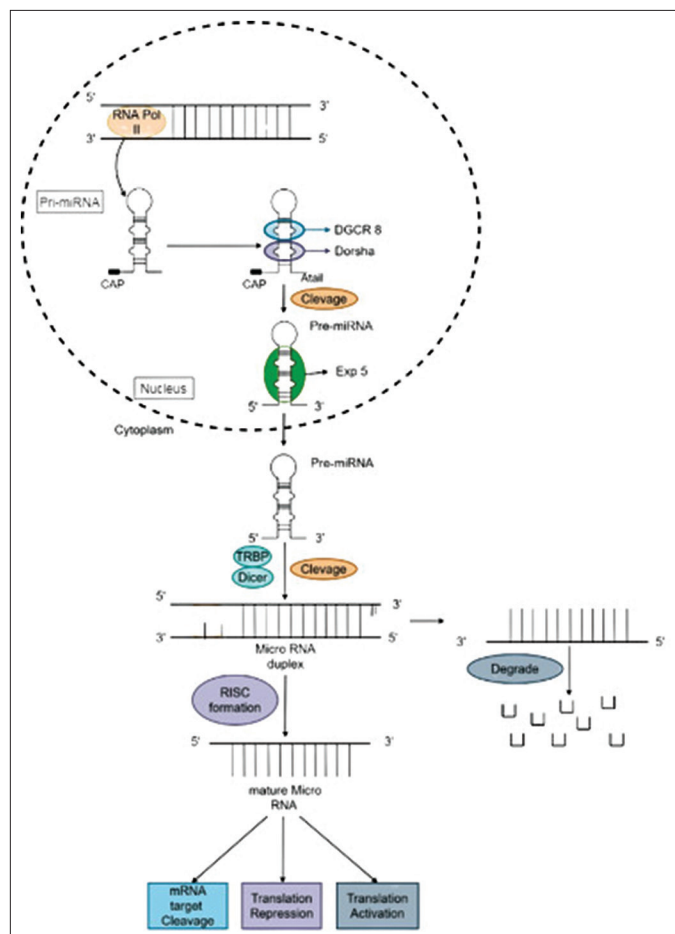

Figure 1: miRNA activation by canonical pathway.

2.2. Non-Canonical Pathway

As shown in Figure 2 the proteins involved in the non-canonical pathways are of different sets of proteins when compared to canonical pathways. Dicer-independent pathway and DGCR8/Drosha-independent pathway are the two types of non-canonical pathways. In DGCR8/Drosha-independent pathway, the pre-miRNA produced will resemble the dicer substrate. During splicing of introns of mRNA, mirtrons are produced which are example of such Pre-miRNA. Through exportin 1 without drosha cleavage, in cytoplasm the nascent RNAs are exported. These miRNA has a 7-methylguanosine cap that will not allow them to load into the Argonaute. Due to dicer substrate's insufficient length in cytoplasm to complete the maturation of pre-miRNAs AGO2 is required. This also promotes loading of all pre-miRNA into AGO2-dependent and AGO2 independent slicing of 3p strand. To complete the maturation of 5p strand 3'-5' trimming is required. Thus these miRNA's could play a key role in the animals including roles in cell apoptosis, grow and cell proliferation [12].

3. miRNA AS PROMISING BIOMARKERS FOR CANCER

miRNA plays an important role in the metabolism, immunity etc. Dysregulation of miRNA expression is closely correlated with tumorogenesis, prognosis and progression of cancer. miRNA can be classified as tumor suppressor and oncogenic miRNA. Mutations, deletions or dysregulation of miR-15/16, miR-34, and miR-200 family can cause colon cancer, multiple myeloma, chronic lymphocytic leukemia, follicular lymphoma, lung cancer, ovarian cancer, GC, bladder cancer, pancreatic cancer, etc. Oncogenic miRNA such as miR-17/92 and miR-222/221 can cause prostate cancer, lung cancer, breast cancer, Ras-induced senescent fibroblast, glioblastoma, non-small cell lung cancer, and hepatocellular carcinoma [13].

Various findings have suggested that biomarkers such as carbohydrate antigen 19-9, carcinoembryonic antigen, carbohydrate antigen 125, and alpha-fetoprotein will improve the responsiveness while diagnosis [7]. miRNA-34a has given us the evidence that it has impact on expression of genes that are associated to apoptosis other than this miRNA-373, miRNA-10b, and miRNA-520c have resulted to encourage tumor metastasis and invasion. miRNA-17-5p/20a inhibits cell apoptosis through cyclin dependent kinase (CDK) inhibitor P21 and the transcriptional modulation of tumor suppressor protein p53INP1 and it upregulates GC cell cycle progression. MiR-93 and miR-106b will hamper the function of BCL 2L11 which is an anti-apoptotic protein by damaging TGF beta [7].

4. miRNA IN GC PROGRESSION AND METASTASIS

As shown in Figure 3 miR-150 has exhibited to influence the GC growth by selecting EGR2 which is a tumor suppressive transcription factor. In most of the cases miR-375 is downregulated but when it is over expressed it will decrease the cell viability [7,14]. MiRNA-146a is ectopically expressed in cancer tissues which inhibits the invasion and migration of the GC cells and affects the EGFR expression. MiRNA-148a and miRNA-152 are correlated with increase in tumor size when the expression are downregulated, while low expression levels of miR-204, miR-146a, or miR-142-5p are correlated with increased tumor size [7,8]. As shown in Table 1, expression of the miRNA-125a-5p is associated with an enhanced malignant potential such as tumor size and depth and poor clinical prognosis. Proto oncogene ERBB 2 is a direct target of miRNA-125a-5p which potently suppresses the proliferation of GC cells. miR-21 is downregulated in GC and it is a well-known for its expression in tumor suppressor

[7]. The most explored single nucleotide polymorphism in the GC are associated with miR-421, miR-608, miR-492, miR-27a, and miR-146a genes. There are 2 miRNA's (miR-155, miR-223) which have exhibited increase in correa's cascade in both the regions, that is, antrum and corpus mucosa [2,9]. VGL4 suppresses proliferation of GC cell by over activation of YAP-TEAD signal and expression pattern of VGL4 and miR-222 is negatively correlated [15]. MiR-204 is negatively correlated with Bcl-2 proteins and increasing in the Bcl-2protein promises for therapeutic and preventive strategy against GC [16,17]. During progression of GC upregulation of miR-181b plays an important role and miR-181b is negatively correlated with metalloproteinase TIMP3 which inhibits the proliferation of tissues [18]. There was significant difference in the level of serum miR-20a in various types of cancer such as GC, breast cancer, nasopharyngeal cancer against non cancerous control. In additional, the level of miR-20a is directly correlated to age, lymph node metastasis, differentiated degree, and tumor stage in GC [19]. Table 1 gives us a brief idea about the list of miRNAs which is associated with cancer.

When HGMA 2 is highly expressed, it has direct connection with the tumor invasion which is contrarily regulated by the let -7 family. When
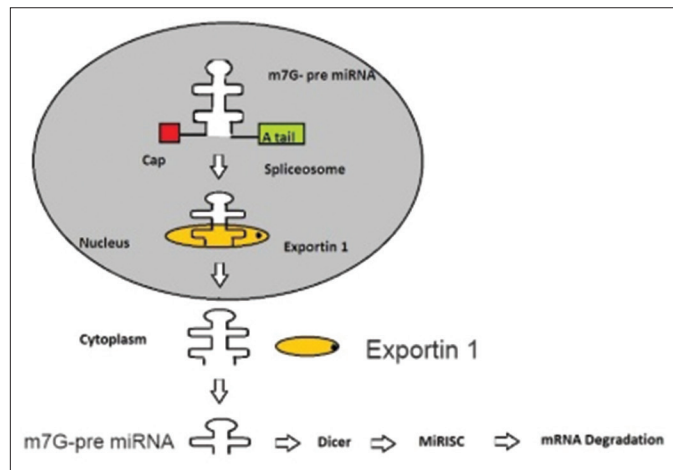

Figure 2: miRNA functional activation by non-canonical pathway.

miR-214regulates the hedgehog signaling pathway, it results in the contribution for GC.

5. microRNA IN BIOLOGICAL FLUIDS

miRNA such as miRNA-106b, miRNA-21, miRNA-106a, and miRNA-17-5p are over expressed in the plasma of GC patients. miR-196a/b has higher specificity in diagnosing the GC when compared with carcinoembryonic antigen and carbohydrate antigen 19-9. Heterogeneity nature of the tumor makes single miRNAs in blood a non ideal technique for the GC diagnosis. That is why to improve their performance for diagnosis of GC combination of plasma miRNA's used. Patients suffering with GC highly expressed miR-21-5p in urine compared with healthy people and after surgical resection of cancer miR-21-5p expression was significantly reduced [23].

MiRNA's have an excellent stability and they can sustain any of the conditions like boiling, freeze-thawing, high/low pH which indicates that these miRNA's can be used as biomarkers. miRNA-129, miRNA-21, miRNA-129-1-3p, miRNA-133a, miRNA-106a, miRNA-421a, etc., are found in the gastric juice and can be used in GC diagnosis in the early stages [Table 2]. MiR-133a, miR-421 levels were lower in the GC patients when compared to normal individuals [23].

6. miRNA IN GC DIAGNOSIS

miRNAs play a key role in the GC diagnosis. It was suggested that there are certain miRNAs which do not operate alone rather they form different clusters with other miRNA and share their functions. As shown in Table 3, miR-21 plays a very important role as it is upregulated in most types of the cancer and it plays a key role in tumorigenesis [8]. In almost 92% human GC, miR-21 is said to be over expressed hence it can be used as a promising biomarker. There are a lot of methods available for medical attention/treatment for GC but some of important therapy such as radiation therapy, surgery, and chemotherapy; however, the detection of the cancer can be done using the miRNA. When miR-181b was transfected into the GC patient it showed increase in cell migration, invasion, and cell proliferation. Hence, this can be used as a potential target for the anticancer therapeutics in GC. miR-7 plays a key role in blocking the movement of tumor cells by targeting the insulin like growth factor-1 receptor [61].
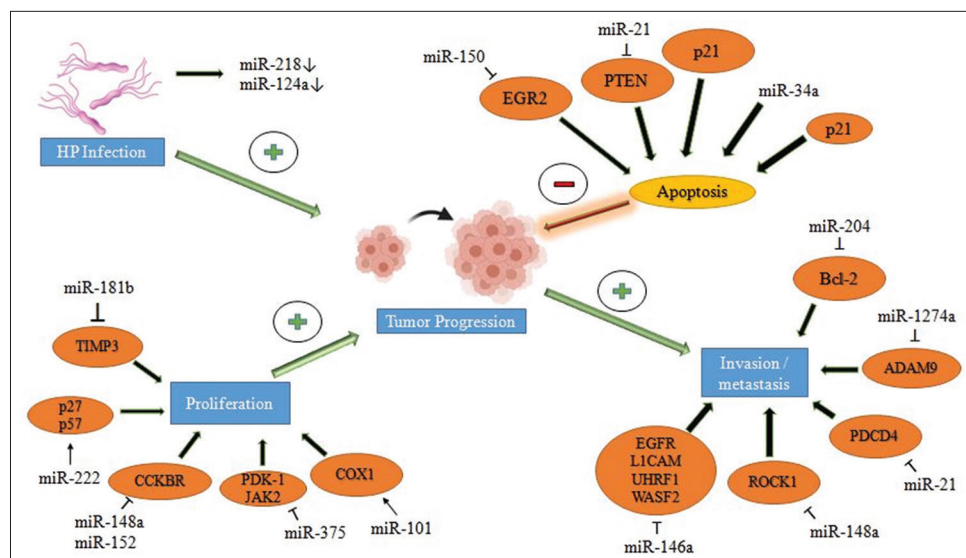

Figure 3: Different microRNA and their role in the tumor progression in Gastric cancer.

Table 1: List of microRNA that are dysregulated in Gastric cancer.

MicroRNA's	Functions	Gene/protein	References
Upregulated miRNA			
miR-17-5p/20a	Cell proliferation and apoptosis	TP53INP1	[20]
miR-125b	Cell proliferation, invasion and migration	PPP1CA	[21]
miR-196a/196b	Cell migration and invasion	Radixin	[22]
miR-199a-5p	Cell migration and invasion	Klotho	[23]
miR-940	Migration and invasion	ZNF24	[24]
miR-106b, miR-93	Cell cycle	E2F1, CDKN1A (p21)	[25]
miR-126	Cell growth and colony formation, apoptosis migration and invasion.	PI3KR2, VEGF-A	[26-29]
miR-25	Cell apoptosis	Bim	[25]
MiR-296-5p	Tumor cell growth	CDX1	[30]
MiR-423-5p	Proliferation and colony formation but suppress invasion in gastric cells	TFF1	[31]
MiR-424	Cell proliferation , invasion, colony formation	LATS1	[32]
Down regulated miRNA			
miR-1	Cell proliferation and migration of tumor cells	VEGF-A, EDN1, MET	[33]
miR-141	Cell proliferation, apotosis and invasion	ZEB1, STAT4	[34,35]
miR-126	Cell growth and colony formation , apoptosis migration and invasion	Crk, SOX2	[26-29]
miR-15a	Cell proliferation, EMT, migration, invasion	Twist1, YAP1	[36]
MiR-185	Chemosensitivity	ARC	[37]
miR-29a	Cell invasion, metastasis	ITGB1	[38]
miR-29c	Cell proliferation, adhesion, invasion, and tumor growth	ITGB1	[39]
mir-146a	Invasion, migration	EGFR, IRAK1	[40]
miR-335	Invasion and metastasis	SP-1, Bcl-w	[41]
miR-34a	Migration, invasion and proliferation	PDGFR, MET	[42]
miR-375	Apoptosis	PDK1	[43]
Let-7b	Invasion and metastasis	ING1	[44]
miR-100	Tumor growth, invasion and metastasis	ZBTB7A	[45]
miR-145	Migration, invasion and angiogenesis	Ets1	[46]
miR-148a	Migration and invasion	ROCK1	[47]
miR-302	Migration and invasion	IL-8	[48]
miR-506	Angiogenesis and EMT	ETS1	[49]
miR-1182	Cell proliferation, migration and invasion	hTERT	[50]
miR-1207-5p/ miR-1266	Cell proliferation, migration and invasion	hTERT	[51]
miR-29a/c	Vascular cell growth, metastasis and tube formation	VEGF	[52]
miR-29b/c	Migration and invasion	DNMT3A	[53]

Table 2: List of miRNA in biological fluids.

MicroRNA	Changes in GC	Source	References
let-7a	Down regulated	Plasma	[54]
let-7g	No changes	Serum	[55]
miR-1	Up regulated	Serum	[56]
miR-103	No changes	Plasma	[57]
miR-106a	Up regulated	Serum	[58]
miR-106a	Up regulated	Serum+exosomes	[59]
miR-106b	Up regulated	Serum	[60]
miR-130a	Up regulated	Serum	[58]
miR-148a	No changes	Serum	[60]
miR-17	Up regulated	Plasma	[54]

When miRNA merge with the target messenger RNA it will give rise to negative regulation of the proteins. We can use this like a secret weapon to up or down regulate the expression of tumor associated genes. When prohibiting is down regulated it will suppress miR-27a which hamper the GC cell growth and even it will support the hypothesis that miR-27a functions as oncogenes. MiR-34 has potential role in the downstream pathways of p53 protein and for some of the target genes like NOTCH, Bcl-2 and HGMA2 where it acts as a potential tumor suppressor as they are entailed in cancer stem cell renewal and survival [8].

The expression of miR-25 was over expressed in GC patients along with lymph node metastasis. When miRNA-25 was inhibited, it repressed the proliferation, metastasis, invasion, and decreased the metastatic space of GC cells by repressing ERBB2,1 expression [85]. MiR-129 plays a key role in regulating cell proliferation by downregulating the

Table 3: Role of miRNA in the proliferation of Gastric Cancer.

MiRNA	Up or down regulation	Regulatory pathway	Inhibit or promote biological function	Related biological function	References
miR-449	Up	Activating p53 pathway	Inhibit	Proliferation	[62]
miR-21	Up	PTEN expression	Promote	Proliferation and invasion	[63,64]
miR-9 and miR-433	Down	GRB2 and RAB34 expression	Promote	Proliferation	[65]
miR-222	Up	P27 and p57	Promote	Proliferation	[66]
miR-375	Up	PDK/Akt signalling pathway	Inhibit	Proliferation	[43]
miR-375	Up	JAK2 oncogene PDK1	Inhibit	Proliferation	[67,68]
miR-9	Up	NF-kB1 gene	Inhibit	Proliferation	[69]
miR-141	Down	FGRFR2 signalling pathway	Promote	Proliferation	[70]
miR-199a	Up	Smad4TGF-β	Promote	Growth and survival	[71]
miR-181a	Up	KLF6 gene	Promote	Proliferation, colony formation, migration and invasion	[72]
miR-181b	Up	CREB1	Inhibit	Proliferation and colony formation	[73]
miR-124	Up	SPHK1	Inhibit	Proliferation and tumorigenicity	[74]
miR-409-3p	Up	PHF10	Inhibit	Proliferation	[75]
miR-182	Up	CREB1	Inhibit	Proliferation and colony formation	[76]
miR-223	Up	FBW7/hCdc4-gene	Promote	Proliferation	[77]
miR-409-3p	Up	RDX	Inhibit	Invasion and metastasis	[78]
miR-27	Up	EMT	Promote	Metastasis	[79]
miR-625	Up	LIMS1-Ilk-parvin	Inhibit	Migration and invasion	[80]
miR625	Down	LIMS1-ILK-parvin	Promote	Migration and invasion	[80]
miR-495 and miR-551a	Up	PRL-3	Inhibit	Migration and invasion	[81]
miR-10b	Up	HOXD10RhoC-ART signalling pathway	Promote	Invasion	[82]
miR-145	Up	N-cadherin protein	Inhibit	Invasion and metastasis	[83]
miR-610	Up	VASP	Inhibit	Migration and invasion	[84]

CDK 6. miRNA-129-2 and miRNA-129-1 significantly showed lower amounts in the GC patients [85]. The microarray profiling with GC patients and normal individuals could collate the expression patterns where the researchers found that miRNA-199a-3p was notably elevated in GC patients. MiR-199a-3p was notably associated with lymph node metastasis, tumor death and stage [85]. miR-196a and miR-196b are upregulated in the gastric tumor tissues. Expression of both miRNA-196b and miRNA-196a is more efficacious than the carbohydrate antigen 19-9 or carcinoembryonic antigen as they have higher specificity and sensitivity [86]. MiR-647 plays a remarkable role in reducing metastasis tumor size as well as it is down regulated in GC patients. When miR-647 is over expressed in the cell lines it suppressed the proliferation of cells and it arrest the cell cycle at G0/G1 stage and induces cell death. Furthermore, in *in vivo* condition it remarkably hamper the growth of tumor. Both *in vivo* and *in vitro* studies have strongly recommended the anti-tumorigenic effects [86].

7. LIMITATIONS WITH CURRENTLY AVAILABLE BIOMARKERS

Till now, there was not any single blood based biomarkers which have enough sensitivity for screening of GC. There are two biomarkers

cancer antigen 72-4 (CA-72-4), carcinoembryonic antigen and cancer antigen 19-9 (CA 19-9) have been associated with GC [2]. Serological biopsy was done to test for the gastric mucosa which was a tumor based biomarker. Concentration of pepsinogen I and II correspond with AG in pre neoplastic condition in intestinal type of GC and it is commonly used in Asian countries. In Europe, the pepsinogen I and pepsinogen II boards are extended by the utilization of Gastrin -17. G-17 is created by the G-cells and stimulates the pepsinogen and hydrochloric acid (HCl) production and consequently it was essential for a similar physiological course as pepsinogen I [2].

When compared with adjacent normal counterparts, in GC tissue 73% (44/60) of miR-421 was overexpressed and there was also no correlation with patients having poor prognosis. This shows that upregulation of miR-421 in GC could be used as diagnostic cancer at early stage. Another group of researchers found that, by real-time PCR, miR-31 was downregulated in GC tissue nevertheless the molecular mechanism has to be explicated. The clinic-pathological features such as differentiation, tumor size, stage, invasion, and metastasis were found to be correlated with the expression of miR-106a. In gastric carcinoma, the higher levels of miR-106a were detected when compared with the non-tumor tissue. Lately, five miRNA signatures with 0.85% as the

receiver operating characteristic curve (miR-34, miR-20, miR-423-5p, miR-1, and miR-27a) were identified for GC as diagnostic markers, demonstrating a higher sensitivity than the conventional marker (CA19-9 or CAE). The miRNA derived from the tumors was thought to be granulated into the circulatory system. Various studies have shown that miRNA's have a differential expression in GC patients compared to normal controls. Many studies found that conventional markers such as CA72-4, CA12-5, CEA, and CA19-9 had lower specificity and sensitivity. These markers represent a new era for circulating miRNA in cancer diagnosis. MiRNA that is deregulated could be used for GC as a diagnostic biomarker, and hopefully in plasma the deregulation of miRNA can be detected, which would aid GC in the early diagnosis. As a result, antagonizing the action of miR by designing individualized treatment for individual's survival would be done at an early stage to improve the survival rate [87].

miRNA AS PROGNOSTIC MARKER

Most of the researchers are in search for the potential use of miRNA's which could be used as prognostic tools other than those diagnostic applications. Nevertheless, these miRNAs must correlate with metastatic potential and clinical outcomes. In terms of cancer phenotypes differentiation profiling of miRNAs has advantage over mRNAs. A recent report on GC shows a prognostic signature which includes three protective miRNAs', that is, miR-126, let-7a and miR-30a-5p, hazard ratio less than 1 and four risk miRNAs, that is, miR-21, miR-338, miR-223, and miR-10b, hazard ratio greater than 1 and was associated with clinical outcomes. In the patients treated with doxifluridine and S-1, low expression of miRNA-18 and miRNA-21 is associated with survival of the patient. Downregulation of miRNA-125a-3p was linked with invasion of tumor, advancement in clinical stage, metastasis, and serves as a prognostic marker for aggressive GC. Gastric cancerous growth in *in vitro* can be detected by abnormal expression of miRNA-125a-3p this tumor suppressive property holds the potential in clinical use. Similarly other miRNA such as miRNA-125-5p and miRNA-125a has same tumor suppressive potential in gastric and reported that, these miRNA's are also expressed low and associated with poor prognosis [87].

8. CONCLUSION

GC ranks third among the cancer related death worldwide despite the advancement in medical field and metastasis has increased over 40% in last two decades. The significance of miRNAs in disease science has been broadly increased till date and lot of attention was given to the miRNA for GC diagnosis. Therefore, the mechanism underlying the progression and metastasis of GC is vital for cancer research. In this context, our review provides an insight into the role of miRNA in GC progression, metastasis and also with an overview of differential expression of miRNA in proliferation of GC. We need a breakthrough in some of the areas of future research to understand the origin of the circulating miRNA and their role in different stages of cancer, its prognosis, early detection, and development of effective therapeutic strategies. This review helps in the identification of miRNA targets for early diagnosis of GC, to improve the survival rate and in the development of therapeutic targets.

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10. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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12. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

13. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects and hence no ethical approval was required.

14. DATA AVAILABILITY

The data generated and analyzed in this review article are included and the reference are cited in the reference section.

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