MicroRNA, the New Genetic Frontier of Gallbladder Carcinoma

Authors:	*Raj Roy,[1] Debojit Das,[1] Chitrita Kundu[1] 1. North Bengal Medical College and Hospital, Siliguri, India *Correspondence to rajroy2632@gmail.com

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Abstract

Introduction: Gallbladder carcinoma (GBC) is an aggressive malignancy characterised by late diagnosis, limited therapeutic options, and poor clinical outcomes. Emerging evidence has identified microRNAs (miRNA) as critical post-transcriptional regulators of gene expression that play pivotal roles in cancer initiation, progression, metastasis, and therapeutic resistance.

Objective: The objective of the study is to assess the prognostic impact of miRNA in GBC, with the goal of improving targeted therapeutic strategies and treatment protocols. The study also aims to identify the current gap in early diagnostic tools and prognostic assessment for GBC.

Methods: The study conducted an electronic search using various databases, including Medline, PubMed, Embase, Cochrane, and Google Scholar, to find articles related to miRNA and GBC.

Results: The study found that miRNAs play a crucial role in regulating gene expression and influencing cellular processes in GBC. The study also identified several miRNAs that have been shown to be involved in GBC, including miRNA-155, miRNA-146b-5p, miRNA-141, miRNA-195-5p, and miRNA-335. The results of the study show that miRNAs play a crucial role in regulating key signalling pathways in GBC, including PI3K/protein kinase B (AKT), Wnt/β-catenin, epidermal growth factor receptor, and JAK/STAT. Additionally, the study found that miRNA-146b-5p causes apoptosis and G1 phase arrest, which are responsible for inhibition of the progression of GBC.

Key Points

1. MicroRNAs (miRNAs) have been identified as potential biomarkers for GBC, with several types of miRNAs showing promise, including miRNA-155, miRNA-146b-5p, miRNA-141, miRNA-195-5p, and miRNA-335.

2. Key findings include the identification of specific miRNAs that regulate key signalling pathways, such as miRNA-21, which suppresses *PTEN* and promotes proliferation, migration, and invasion in GBC. Additionally, miRNA-145-5p is downregulated in tumour tissues and direct and indirect signal transducer and activator of transcription 1 target genes are activated when miRNA-145-5p is expressed.

3. Constitutive activation of signal transducer and activator of transcription 3 has been reported in GBC and is associated with poor prognosis and advanced disease stage.

INTRODUCTION

Gallbladder carcinoma (GBC) is recognised as one of the most lethal and aggressive malignancies affecting the biliary tract.[1] Although gallbladder cancer and cholangiocarcinoma are often grouped together in broader 'biliary tract cancer' statistics, retrospective epidemiological data demonstrate that they remain distinct clinical entities and separate cancer sites. Worldwide, around 2.3 per 100,000 individuals are diagnosed with GBC.[2] It accounts for 1.2% of all cancer diagnoses and 1.7% of all cancer deaths, making it the 22nd most common cancer and 17th most deadly malignancy.[2] According to the GLOBOCAN 2022 observational data specifically for GBC (reported separately from cholangiocarcinoma), there are 122,491 new cases per year, with 89,055 deaths annually.[3]

Anatomically, the gallbladder is a small cystic organ that is involved in the storage and concentration of bile. It is located under the inferior surface of the liver. The risk factors that have been implicated in the aetiology of GBC are obesity, ethnicity, age, chronic inflammation, gallstones, viral hepatitis, exposure to heavy metals and environmental toxins, gallbladder polyps, genetic predisposition, and anatomical abnormalities. These abnormalities induce metaplastic changes in gallbladder epithelium, initiating a pathological cascade that progresses from dysplasia to carcinoma *in situ*, and ultimately invasive carcinoma.[4] Adenocarcinoma, papillary carcinoma, squamous carcinoma, and adeno-squamous carcinoma are several subtypes of this disease. It is characterised by rapid progression and asymptomatic onset, which leads to it being diagnosed at advanced stages, thereby complicating treatment outcomes. Recent studies show that the 5-year survival rate is over 90% for Stage I, while it is around 30% for Stage III and IV, based on standard American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) staging criteria.[5,6] Currently, surgical resection remains the only definitive curative option available for patients diagnosed with GBC.[7] In response to these challenges, researchers and clinicians are concentrating on biomarker molecules, which can provide critical insights into the presence, progression, and treatment response of a disease. Early diagnosis, prediction of outcomes, and ideas about clinical treatment can be made possible through biomarkers, revolutionising the field of GBC.

The biomarker was initially referred to as "biochemical marker" by Mundkur in 1949, and in 1957, Porter referred to it as "biological marker."[8,9] The term biomarker was formally defined in 1973 as "the presence or absence of biological material."[10] In 2000, the National Institutes of Health (NIH) Biomarkers Definitions Working Group proposed a definition of 'biomarker' that was widely accepted as an international definition of biomarker in clinical pharmacology.[11] The FDA also defined biomarkers according to their own perspective, classifying them into distinct categories such as diagnostic, prognostic, predictive, safety, and monitoring biomarkers to standardise their use in

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evaluating the safety and efficacy of medical treatments and guiding regulatory decision-making.[12] Taken together, biomarkers can be defined as a particular component that is associated with a normal biological process, pathological mechanism, biological response to external interference, chemical agent, or a group of chemical agents, but not the presence of an agent or its metabolite within the body tissues.[13,14]

In the case of GBC, microRNAs (miRNA) have emerged as promising candidate biomarkers for prognostic evaluation. They are approximately 22 nucleotides long, transcribed from DNA into RNA hairpins, and regulate gene expression post-transcriptionally by binding to the three prime untranslated region (3'-UTR) of target mRNAs.[15] While numerous miRNAs have been implicated in GBC across preclinical *in vitro* models and observational clinical cohorts, the authors prioritised the discussion of miRNA-155, miRNA-146b-5p, miRNA-141, miRNA-195-5p, and miRNA-335 due to the comparatively higher volume of published evidence supporting their potential relevance (Table 1). miRNA-155 is frequently documented across retrospective human tissue studies evaluating its role in prognosis.[16] Similarly, miRNA-146b-5p has been extensively evaluated in both *in vitro* cell culture models and retrospective tissue cohorts as both an emerging prognostic biomarker and a therapeutic target in GBC.[6,10] miRNA-141 has been characterised in multiple *in vitro* reports regarding its involvement in cell proliferation and epithelial–mesenchymal transition (EMT), as well as human retrospective studies for prognostic significance. Furthermore, miRNA-195-5p is highlighted in pre-clinical cell line assays and animal models for its therapeutic potential in suppressing key oncogenic signalling pathways, including Wnt/β-catenin. Finally, miRNA-335 has been consistently reported in retrospective clinical studies concerning its prognostic value and *in vitro* models demonstrating its influence on chemoresistance. These five miRNAs collectively represent the most rigorously studied candidates in GBC literature, with data spanning human tissue, circulating markers, and prognostic assessment. miRNA-155 is derived from the processing of B cell integration cluster, which is a non-coding transcript expressed in activated B cells, T cells, monocytes, and macrophages.[16]

Table 1: miRNA candidates with robust evidence from human GBC studies.

miRNA	Type	Evidence base	Potential clinical relevance
miRNA-155	Oncogenic	Retrospective human tissue studies	Prognostic marker
miRNA-146b-5p	Oncogenic	Retrospective tissue cohorts	Prognostic marker/therapeutic target
miRNA-141	Oncogenic	Retrospective human studies	Prognostic marker
miRNA-335	Tumour suppressor	Retrospective clinical studies	Prognostic marker

GBC: gallbladder carcinoma; miRNA: microRNA.

The human genome contains two miRNA-146 genes (*miRNA-146a* and *miRNA-146b*) located on chromosomes 5 and 10, respectively. miRNA-146b-5p is one of the mature miRNAs derived from its precursor (pre)-miRNA-146b.[17] miRNA-141, a member of the miRNA-200 family, is involved in cancer stem cell formation

and regulation of EMT.[18] *In vitro* functional assays demonstrate that migration, invasion, and proliferation are inhibited by miRNA-141 in head and neck squamous cell carcinoma, non-small cell lung cancer, female reproductive cancers, and renal cell carcinoma, but it enhances proliferation and drug resistance in cell line models of colorectal cancer and ovarian cancer.[19-21] miRNA-195 belongs to the miRNA-15/107 family, and this miRNA is responsible for inducing stress and activation in various diseases, such as cancers, heart failure, and schizophrenia.[22] miRNA-195-5p, a 'guide strand', is produced from the hairpin of miRNA-195, which exhibits its function in cell culture and animal models by targeting the mRNAs of multiple proteins as oncogenes or tumour suppressors in several cancers, including breast cancer, lung cancer, prostate cancer, gastric cancer, colorectal cancer, hepatocellular cancer, and bladder cancer.[22-27] miRNA-335 is transcribed from a genomic region on chromosome 7q32.2, and it acts as an oncogenic or tumour suppressor miRNA in various human malignancies.[28] Retrospective tissue analyses show it is upregulated in meningioma, glioma, and myeloma, but downregulation of this miRNA is also observed in retrospective studies of various malignancies, including breast cancer, hepatocellular carcinoma, and pancreatic adenocarcinoma.[29-34]

For prognostic evaluation of GBC, these miRNAs may be valuable. This manuscript is a narrative review with systematic search components; while a structured selection process was used, the synthesis remains narrative and mechanistic. This study aims to assess the prognostic impact of miRNA in GBC so that targeted therapeutic strategies and treatment protocols can be improved by the clinicians. From this study, the current gap in early diagnostic tools and prognostic assessment for GBC can be effectively detected.

METHODOLOGY

The authors conducted an electronic search using various databases (Medline, Ovid, PubMed, Embase, Cochrane, and Google Scholar) for related studies that were published between 1st January 2016–28th February 2026 (Supplementary Figure 1). The search strategy was systematic to ensure comprehensive coverage, but the synthesis is qualitative and narrative in nature, intended to map conceptual relationships rather than provide a formal quantitative meta-analysis. The goal was to find articles related to keywords such as 'microRNA', 'gallbladder carcinoma', and 'GBC', in conjunction with 'prevalence', 'diagnosis', 'pathophysiology', 'therapeutic measures', and 'prognosis'. This search focused solely on studies involving humans, without any language restrictions. Moreover, the authors manually checked reference lists from relevant studies to see how the information applied to their research. To prevent overlap in patient groups, if authors wrote about the same cohorts in multiple publications, only the most recent or detailed study was included.

Data Extraction and Methodological Assessment

The retrieved reports contained information such as authorship, publication year, journal name, sample size, methodologies used, histological information, diagnostic techniques, and therapeutic strategies employed. To minimise bias during data abstraction, four reviewers independently gathered the required data.

While formal quality and risk-of-bias assessments were not conducted in this study, the authors acknowledge this limitation and highlight the need for systematic evaluation in future updates. To ensure rigorous methodological appraisal across the diverse body of evidence, future assessments should incorporate validated critical appraisal tools, such as the Newcastle–Ottawa Scale (NOS) for observational cohort and case-control studies, and the Cochrane Risk of Bias (RoB) 2 tool for RCTs.

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MOLECULAR MECHANISMS GOVERNING miRNA FORMATION AND ACTIVITY

Multifaceted Roles of miRNAs in Cellular Homeostasis

miRNAs are endogenous, small non-coding RNAs that regulate gene expression at the post-transcriptional level. While frequently binding to complementary sequences in the three prime untranslated regions of target mRNAs to induce translational repression or mRNA degradation, miRNAs can also interact with binding sites outside of canonical 3′UTRs, and repression can occur through multiple mechanisms *in vitro*.[35] In GBC, experimental cell line models demonstrate that aberrant miRNA expression disrupts cellular homeostasis by influencing fundamental biological processes including cell proliferation, apoptosis, EMT, invasion, and metastasis.[35] Several retrospective studies have demonstrated distinct miRNA expression signatures in human GBC tissues and serum, indicating that miRNAs can function as oncogenes or tumour suppressors depending on cellular context and target specificity.[36]

Molecular Events Underlying miRNA Biogenesis

miRNA biogenesis is a highly regulated multistep process that begins with transcription of primary miRNA transcripts by RNA polymerase II. These primary miRNAs are processed in the nucleus by the drosha–DGCR8 complex to generate pre-miRNAs, which are subsequently exported to the cytoplasm by exportin-5.[37] Cytoplasmic processing by the ribonuclease III enzyme dicer yields mature miRNA duplexes, one strand of which is incorporated into the RNA-induced silencing complex to mediate gene silencing.[37] Although direct evidence of altered miRNA biogenesis machinery in GBC is limited, dysregulation of this conserved pathway may contribute to the global miRNA expression changes observed in GBC. Despite this limited evidence regarding the biogenesis machinery itself, the pursuit of miRNAs in GBC is strongly justified by the robust, consistent differential expression profiles observed in human patient tissues and the validated functional roles these molecules play in modulating critical oncogenic signalling pathways, such as Wnt/β-catenin, epidermal growth factor receptor (EGFR), and PTEN/PI3K/protein kinase B (AKT), in both *in vitro* and *in vivo* models.

Canonical miRNA biogenesis pathway

In the canonical miRNA biogenesis pathway, sequential cleavage by drosha and dicer ensures accurate maturation of miRNAs that subsequently guide RNA-induced silencing complex to target mRNAs; disruption of canonical miRNA processing has been shown in multiple cancers to result in imbalanced expression of oncogenic and tumour-suppressive miRNAs.[38] Similar perturbations are likely to occur in GBC, contributing to malignant transformation and tumour progression through altered post-transcriptional gene regulation.

Non-canonical miRNA biogenesis pathway

The non-canonical miRNA biogenesis pathway is of two types: a) the drosha/DGCR8-independent pathway and b) the dicer-independent pathway. Mirtrons are pre-miRNA, produced from the drosha/DGCR8-independent pathway.[39,40] 7-methylguanosine capped pre-miRNA is another example of pre-miRNA produced from this pathway. On the other hand, miRNAs that are produced from the dicer-independent pathway require argonaute RISC catalytic component 2 (AGO2) for the completion of their maturation because of their insufficient length.[41]

miRNA-MEDIATED MODULATION OF KEY SIGNALLING CASCADES IN GBC

Wnt/β-Catenin Signalling Under miRNA Control

The Wnt/β-catenin signalling pathway is a critical regulator of cell proliferation, differentiation, and EMT, and is frequently dysregulated in GBC.[42] *In vitro* mechanistic studies indicate that miRNAs can modulate

this pathway by targeting β-catenin itself, or upstream and downstream regulatory components, thereby influencing transcription of oncogenic target genes.[43] Experimental studies in GBC cell lines and *in vivo* xenograft models have demonstrated that miRNA-195-5p suppresses proliferation, migration, and invasion of GBC cells by regulating Wnt/β-catenin signalling, highlighting a direct role for miRNA-mediated regulation of this pathway in GBC progression and showing that it also targets *FOSL1* (Supplementary Table 1)[44] Again, *in vitro* functional assays demonstrate a possible action of miRNA-182 on *FOXN3*, which results in inhibition of the Wnt/β-catenin pathway by competing with transcription factor 4 (TCF4) for binding to β-catenin.[45]

Post-Transcriptional Control of p53 Signalling by miRNAs

The tumour suppressor p53 pathway plays a central role in maintaining genomic integrity by inducing cell cycle arrest, senescence, or apoptosis in response to cellular stress.[46] Retrospective observational studies show that alterations in p53 signalling are common in GBC and are associated with aggressive tumour behaviour. *In vitro* experimental models have shown that microRNAs can modulate p53 signalling by targeting p53 itself or its downstream effectors involved in apoptosis and cell cycle control.[47] Although most mechanistic insights arise from *in vitro* and *in vivo* studies in other malignancies, similar miRNA-mediated post-transcriptional regulation of p53-associated genes is likely to contribute to gallbladder carcinogenesis. Mouse double-minute 2 (*MDM2*) and mouse double-minute 4 (*MDM4*) are p53 regulators that are also targeted by some miRNAs to control p53 indirectly in cell models. Increasing the expression of p53 and p21 and lowering the expression of cyclin D1 are two mechanisms by which miRNA-133a-3p prevents cell growth *in vitro*. In retrospective human GBC tissue analyses, miRNA-133a-3p is expressed at a significantly low level.[48]

Regulation of KRAS-Driven Oncogenic Signalling by miRNAs

Kirsten rat sarcoma viral oncogene homolog (KRAS)-driven oncogenic signalling activates downstream pathways such as MAPK and PI3K/AKT, promoting tumour cell proliferation and survival.[49]

Direct evidence in human GBC

Genomic profiling in human retrospective cohort studies has identified aberrant activation of RAS pathway components in a subset of GBC. A let-7 miRNA binding site polymorphism in the *KRAS* 3′-UTR has been associated with increased risk and reduced survival for GBC in prospective and retrospective human cohort studies.[50] Furthermore, impaired expression of miRNA-143, miRNA-145, and miRNA-200c is frequently observed in human retrospective tissue studies of patients with GBC.[51,52]

Experimental evidence in GBC models

In vitro cell culture and *in vivo* animal model studies specifically investigating direct miRNA–*KRAS* interactions in GBC models remain limited. However, downregulatory patterns of miRNA-200c, miRNA-143, and miRNA-145 in retrospective GBC tissues correlate with altered downstream signalling components of the KRAS cascade.[51,52]

Evidence from biliary tract cancers

In broader biliary tract malignancies, miRNA dysregulation frequently intersects with KRAS pathway activation, influencing tumour aggressiveness and patient prognosis across cholangiocarcinoma and related biliary neoplasms.[51]

Evidence from other cancers

Substantial evidence from *in vitro* and animal model studies in other cancer types demonstrates that miRNAs can directly target *KRAS* transcripts or regulate downstream signalling molecules to suppress MAPK and PI3K/AKT cascades.[50] In particular, functional *in vitro* studies in gastrointestinal and lung cancer cell lines demonstrate that miRNA-200c, miRNA-143, and miRNA-145 directly target *KRAS* to attenuate oncogenic cell growth and survival.[51,52]

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Hypothesised mechanisms requiring validation

It is hypothesised that miRNA-mediated post-transcriptional regulation of *KRAS* transcripts serves as a primary brake on oncogenic driver pathways in GBC epithelium. Direct validation through reporter assays and rescue experiments in human GBC cell lines is required to confirm these specific binding interactions.

PTEN/PI3K/AKT Axis Under miRNA Control

The PTEN/PI3K/AKT signalling axis is a key regulator of cell survival, proliferation, and metabolism, and is frequently dysregulated in GBC.[53] PTEN functions as a tumour suppressor by negatively regulating PI3K/AKT signalling, and loss of PTEN activity results in enhanced oncogenic signalling.[54] *In vitro* models have shown that miRNAs regulate this pathway by targeting *PTEN* or downstream components of the PI3K/AKT cascade. Dysregulation of miRNAs controlling this axis may therefore contribute to enhanced tumour growth, invasion, and resistance to apoptosis in GBC. According to the cell culture study of Yang et al., miRNA-21 suppresses *PTEN* and helps aquaporins in proliferation, migration, invasion, and prognosis of GBC.[55]

JAK/STAT Pathway Modulation by miRNAs in Gallbladder Cancer

The JAK/signal transducer and activator of transcription (STAT) signalling pathway transduces extracellular cytokine signals to the nucleus and regulates processes such as proliferation, inflammation, and immune evasion.[56]

Direct evidence in human GBC

Constitutive activation of STAT3 has been reported in retrospective human GBC tissue samples and is significantly associated with poor prognosis, advanced disease stage, and vasculogenic mimicry.[57] Furthermore, miRNA profiling of human GBC tissues in retrospective cohorts reveals distinct miRNA profiles, including significant downregulation of miRNA-145-5p compared to adjacent normal tissue.[58]

Experimental evidence in GBC models

In vitro restoration of the tumour-suppressive miRNA-145-5p in GBC cell models leads to the activation of direct and indirect STAT1 target genes, thereby modulating inflammatory and tumour-suppressive transcriptional responses.[58]

Evidence from biliary tract cancers

Across biliary tract cancers, cytokine-driven JAK/STAT activation promotes invasive tumour phenotypes and modulates the inflammatory microenvironment, frequently operating under the epigenetic control of miRNA clusters.[56]

Evidence from other cancers

In vitro and animal model studies in other solid malignancies indicate that miRNAs can directly modulate the JAK/STAT pathway by targeting JAKs, STAT transcription factors, or upstream cytokine receptors, thereby inhibiting cell proliferation and immune evasion.[56]

Hypothesised mechanisms requiring validation

Although specific miRNAs directly targeting JAK or *STAT3* in GBC remain to be fully characterised, it is hypothesised that dysregulated miRNAs contribute to persistent STAT3 phosphorylation and downstream immune evasion in gallbladder carcinogenesis, a mechanism requiring targeted functional validation.

miRNA-Mediated Modulation of EGFR Signalling in GBC

EGFR signalling plays a pivotal role in GBC by promoting cell proliferation, survival, and invasion through activation of downstream PI3K/AKT and MAPK pathways.[59] Overexpression of *EGFR* has been documented in retrospective GBC human tissue studies and is associated with aggressive tumour behaviour.[60] *In vitro* models across various cancers have shown that microRNAs regulate *EGFR* expression and signalling activity, thereby influencing tumour growth and therapeutic response. Although direct evidence in GBC human clinical trials is absent, existing pre-clinical data suggest that miRNA-mediated modulation of EGFR signalling represents

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an important regulatory mechanism in GBC pathogenesis. *In vitro* assays demonstrate that miRNA-146b-5p causes apoptosis and G1 phase arrest, which are responsible for inhibition of the progression of GBC cell lines. It is shown in cell culture that miRNA-146b-5p acts by targeting the EGFR signalling pathway, and ectopic *EGFR* expression can reverse this action of miR-146b-5p.[61]

THERAPEUTIC TARGETING OF miRNAS IN GALLBLADDER CANCER

Rationale for miRNA-Based Therapeutic Strategies

In case of GBC, surgical resection is the only curative treatment. GBC is highly susceptible to liver invasion and metastasis.[62] miRNAs represent attractive therapeutic targets in GBC due to their ability to simultaneously regulate multiple oncogenic and tumour suppressor pathways involved in tumour initiation, progression, and metastasis. Aberrant expression of specific miRNAs in GBC contributes to dysregulation of key signalling networks such as PI3K/AKT, Wnt/β-catenin, EGFR, and JAK/STAT pathways, suggesting that restoring normal miRNA expression may reverse malignant phenotypes.[63] Unlike single-gene targeted therapies, miRNA-based interventions offer a broader regulatory scope, potentially overcoming pathway redundancy and therapeutic resistance observed in GBC.[64,65]

miRNA Replacement Therapy Using Tumour Suppressor miRNAs

Tumour suppressor miRNAs that are downregulated in retrospective patient studies represent promising candidates for miRNA replacement therapy. Restoration of these miRNAs using synthetic miRNA mimics has been shown in preclinical *in vitro* and *in vivo* animal models to inhibit tumour cell proliferation, induce apoptosis, and suppress invasion and metastasis. For example, miRNA-195-5p has been demonstrated in cell lines to inhibit gallbladder cancer cell growth and migration through suppression of Wnt/β-catenin signalling, indicating that reintroduction of such miRNAs may exert therapeutic benefits. miRNA-335, miRNA-122, miRNA-26a, miRNA-135a-5p, miRNA-29b, and miRNA-200a also act as tumour suppressor miRNAs in pre-clinical models. Hence, stimulation of these miRNAs may represent a promising therapeutic strategy in GBC that warrants evaluation in clinical trials. *In vitro*, overexpression of miRNA-335 results in the decrease of ATP binding cassette transporter B1 and ATP binding cassette G2, and inhibition of viability of GBC cells when treated with 5-fluorouracil of various concentrations, thereby increasing 5-fluorouracil sensitivity.[66] Histone deacetylase inhibitor PCI-24781 acts as a therapeutic material in pre-clinical GBC models; in cell culture, PCI-24781 increases the amount of miRNA-122 that is downregulated in GBC.[67] In cell culture experiments, miRNA-26a reduced GBC cell proliferation by arresting the cell cycle at the G1/S phase.[68] Hence, miRNA-26a warrants further investigation as a potential therapeutic candidate. *In vitro* and *in vivo* animal experiments showed that miRNA-135a-5p hinders GBC cell growth by increasing caspase 3/7 and inducing G1/S cell cycle arrest.[69] Cell line experiments showed that stimulation of miRNA-29b and miRNA-200a is related to the silencing of an oncogenic factor, aquaporin 5 (*AQP5*), by small interfering RNA, indicating that both of these miRNAs may represent therapeutic candidates in GBC.[70] These pre-clinical findings support the feasibility of miRNA replacement strategies to restore tumour suppressive regulatory networks in GBC. All the preclinical therapeutic approaches of these tumour suppressor miRNAs are summarised in Table 2.

Inhibition of Oncogenic miRNAs Using AntagomiRs

Oncogenic miRNAs (oncomiRs) that are overexpressed in retrospective human GBC studies can be therapeutically targeted using antisense oligonucleotides, antagomiRs, or locked nucleic acid inhibitors. These approaches aim to neutralise oncogenic miRNA activity, thereby derepressing tumour suppressor genes and inhibiting oncogenic signalling pathways. Preclinical *in vitro* and *in vivo* animal studies in hepatobiliary and gastrointestinal malignancies have shown

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Table 2: Role of tumour suppressor miRNAs in GBC therapeutic approaches.

miRNAs	Therapeutic intervention	Mechanism of therapeutic effect
miRNA-195-5p	Stimulation (overexpression)	Inhibits proliferation, migration, and invasion of GBC cells by regulating the Wnt/β-catenin signalling pathway, also targeting *FOSL1*
miRNA-335	Stimulation (overexpression)	Increases the sensitivity of GBC cells to 5-fluorouracil, which causes inhibition of the viability of GBC cells
miRNA-122	Stimulation (overexpression)	Histone deacetylase inhibitor PCI-24781 upregulates this miRNA
miRNA-26a	Stimulation (overexpression)	Reduces cell proliferation due to G1/S cell cycle arrest by targeting *HMGA2*
miRNA-135a-5p	Stimulation (overexpression)	Restricts GBC cell growth both *in vivo* and *in vitro* by increasing caspase 3/7 activity and inducing G1/S cell cycle arrest
miRNA-29b	Stimulation (overexpression)	This tumour suppressor microRNA is upregulated due to silencing of *AQP5* by siRNA
miRNA-200a	Stimulation (overexpression)	This tumour suppressor miRNA is upregulated due to silencing of *AQP5* by siRNA

GBC: gallbladder carcinoma; miRNA: microRNA; siRNA: small interfering RNA.

that inhibition of miRNA-21 and miRNA-155 reduces tumour growth, invasion, and chemoresistance, suggesting similar therapeutic potential in gallbladder cancer. Histone deacetylase inhibitor PCI-24781 downregulates miRNA-21, miRNA-142-3p, miRNA-142-5p, and miRNA-223 in cell models; these miRNAs are oncogenic in nature. Another oncogenic miRNA identified in pre-clinical assays is miRNA-182, which mainly targets cell adhesion molecule 1.[71] *In vitro* suppression of miRNA-182 reduces the migration and invasion of cancer cells stimulated by TGF-β.[71] *In vivo* animal experiments confirm that downregulation of miRNA-182 also prevents pulmonary metastasis.[71] Targeting oncomiRs may thus provide an effective approach for mitigating malignant development in GBC, though clinical trials are required to establish safety and efficacy in humans. All the pre-clinical therapeutic approaches of these oncogenic miRNAs are summarised in Table 3.

Delivery Strategies for miRNA-Based Therapeutics

Efficient and targeted delivery remains a major challenge for miRNA-based therapies. Various delivery systems, including lipid nanoparticles, viral vectors, exosomes, and polymer-based carriers, have been explored to enhance miRNA stability, cellular uptake, and tissue specificity.[72] In the context of GBC, liver-directed and hepatobiliary-targeted delivery platforms are particularly relevant due to anatomical proximity and shared vasculature. Progress in nanotechnology and exosome-mediated transport has enhanced the practicality of systemic miRNA treatment, reducing off-target effects and immune activation.

miRNAs and Sensitisation to Chemotherapy and Targeted Therapy

Emerging evidence from *in vitro* and *in vivo* animal models suggests that miRNAs play a critical role in modulating therapeutic response and drug resistance in GBC; dysregulated miRNAs can influence chemosensitivity in cell lines by regulating apoptosis, DNA repair mechanisms, and drug efflux transporters.[73] Restoration or

Table 3: Role of oncogenic miRNAs in GBC therapeutic approaches.

miRNAs	Therapeutic intervention	Mechanism of therapeutic effect
miRNA-21	Suppression (downregulation)	Histone deacetylase inhibitor PCI-24781 downregulates this miRNA
miRNA-142-3p	Suppression (downregulation)	Histone deacetylase inhibitor PCI-24781 downregulates this miRNA
miRNA-142-5p	Suppression (downregulation)	Histone deacetylase inhibitor PCI-24781 downregulates this miRNA
miRNA-223	Suppression (downregulation)	Histone deacetylase inhibitor PCI-24781 downregulates this miRNA
miRNA-182	Suppression (downregulation)	Migration and invasion of cancer cells stimulated by TGF-β is reduced due to suppression of miRNA-182; downregulation of miRNA-182 inhibits pulmonary metastases *in vivo*

GBC: gallbladder carcinoma; miRNA: microRNA.

inhibition of specific miRNAs has been shown in pre-clinical cell line and animal studies to enhance sensitivity to chemotherapeutic agents and targeted therapies by suppressing survival pathways such as PI3K/AKT and EGFR signalling. Therefore, miRNA-based therapeutic strategies may also function as adjuvant approaches to improve the efficacy of existing treatment modalities in GBC, pending validation in human prospective studies and clinical trials.

Challenges and Future Directions in miRNA Therapeutics for GBC

Despite promising pre-clinical data from cell lines and animal models, no miRNA-based therapeutics for GBC have yet advanced through human clinical trials. Key challenges hindering clinical translation include issues related to delivery specificity, off-target effects, immune activation, and long-term safety.[74] Additionally, tumour heterogeneity observed in human observational cohorts complicates patient stratification and therapeutic design. Future research should focus on validating GBC-specific miRNA signatures in prospective human cohorts, optimising delivery platforms *in vivo*, and initiating early-phase clinical trials to evaluate miRNA therapeutics within precision medicine frameworks.

FUTURE OUTCOMES

Earlier investigations into GBC have established the critical involvement of miRNAs in tumourigenesis, progression, and therapeutic resistance through modulation of key oncogenic and tumour suppressor pathways, including PI3K/AKT, Wnt/β-catenin, EGFR, and p53 signalling.[63] Nevertheless, the majority of existing studies have examined individual miRNAs or discrete signalling axes in isolation. The present work addresses this limitation by providing an integrative narrative synthesis of miRNA dysregulation, linking molecular mechanisms with diagnostic and therapeutic relevance within a unified conceptual framework. While pathways are synthesised here based on current literature, this conceptual integration highlights potential convergent regulatory patterns rather than serving as a definitive systems-level map. Consequently, performing rigorous systems biology network analysis remains a key priority for future research. Such insights may guide future functional investigations, enable the development of multi-miRNA diagnostic panels with enhanced clinical performance, and support the rational design of miRNA-based therapeutic strategies including miRNA mimics, antagomiRs, and combination approaches with targeted agents to improve treatment stratification and overcome therapeutic resistance in GBC.

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CONCLUSION

In conclusion, while individual miRNAs have been extensively investigated in GBC, comprehensive systems-level mapping of their interactions remains a challenge. This review provides a synthesis of existing literature to propose conceptual relationships between miRNA dysregulation and key oncogenic pathways, offering a framework for future hypothesis generation rather than a definitive demonstration of novel interactions. These narrative syntheses highlight potential regulatory overlaps, but rigorous analytical and experimental validation is required to confirm these complex networks. Future studies, including dedicated systems biology modelling, will be essential to substantiate these conceptual frameworks and advance miRNA-based strategies towards clinical application.

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