MicroRNA expression and its implications for diagnosis and therapy of gallbladder cancer

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ABSTRACT

Gallbladder cancer is the most common biliary tract malignancy with poor prognosis. MicroRNAs (miRNAs) are a class of small, endogenous, non-coding RNAs of 19–23 nucleotides in length, which regulate gene expression at post-transcriptional and translational levels. Several studies have demonstrated aberrant expression of miRNAs in gallbladder cancer tissues. Recent evidences also demonstrated that specific miRNAs are functionally involved in gallbladder cancer development through modulating cell proliferation, apoptosis, migration, invasion and metastasis. In this review, we explore the possibilities of using miRNAs as prognostic, diagnostic markers and therapeutic targets in gallbladder cancer.

INTRODUCTION

Gallbladder cancer is the most common biliary tract malignancy with poor prognosis and the fifth most common gastrointestinal malignancy worldwide [1-3]. The 5-year survival rates of advanced-staged GBC patients ranges from 20% to 40% [4-7]. Gallbladder cancer is usually diagnosed at advanced stage due to absence of specific symptoms [8-10]. Despite recent advances in its diagnostic techniques and therapeutic managements that might give hope on consequent disease remission, prognosis of patients with gallbladder cancer remains poor [11-13]. It is therefore of paramount importance to elucidate its molecular biology, genetic causes, and cellular origin in order to develop novel therapeutic strategies to improve clinical outcome of patients with gallbladder cancer [14-17].

MicroRNAs (miRNAs) are evolutionarily conserved, short, endogenous, single-stranded non-coding RNA molecules [18-24]. Through binding to the 3'-untranslated regions (3'-UTRs) of target mRNAs, miRNAs suppress gene expression by inducing mRNA degradation or inhibiting protein translation depending on the degree of sequence complementarity [25-30]. Aberrant miRNA expressions have been reported in various cancers, such as lung, bladder, gastric, nasopharyngeal, breast, liver and pancreatic cancers [31-41]. Moreover, many studies have indicated that miRNAs can play critical roles in modulating cell proliferation, migration, invasion, apoptosis, radio- and chemosensitivity and cancer stem cell phenotype [42-48]. However, miRNAs expression and their implications for diagnosis and therapy of gallbladder cancer remain elusive. In this review, we focus on miRNAs involved in gallbladder development and discuss the potential use of miRNAs as prognostic biomarkers and
treatment strategies for gallbladder cancer.

FUNCTION AND BIOGENESIS OF MiRNA

Previous studies have delineated the molecular basis of miRNA biogenesis, which consists of several steps [49, 50]. It begins with the transcription of primary miRNA (pri-miRNA), which is several-kilobase-long, from a miRNA gene. The pri-miRNA is then excised into one or more approximately 70-nucleotide stem-loop structures to form the miRNA precursors (pre-miRNAs) [51]. Then, the Ran-GTP-dependent nuclear export factor exportin-5 transported pre-miRNA into the cytoplasm, where Dicer processes the pre-miRNA into an approximately 22-nucleotide RNA duplex [52]. Both the mature miRNA strand and its complementary strand are located in the 22-nucleotide RNA duplex [53, 54]. The complementary strand of miRNA is then degraded [55]. By incorporating into the RNA-induced silencing complex (RISC) and binding to the complementary sequences in 3′-UTRs of target mRNAs, miRNA exerts its function through inducing mRNA cleavage or translational inhibition [56, 57].

MiRNAs in Gallbladder Cancer

The first study on the potential association between genetic variations of miRNA genes and susceptibility to gallbladder cancer was published by Srivastava et al. [58]. This case-control study evaluated the potential association of three single nucleotide polymorphisms (SNPs), namely rs2910164, rs11614913 and rs3746444, with risk for gallbladder cancer in 230 cases and 230 controls in a North Indian population. A non-significant increased risk was observed between carriers of variant genotypes of rs2910164, rs11614913 and rs3746444 (odds ratios=1.3, 1.3 and 1.1, respectively). Their data showed that common miRNA variants may not contribute to gallbladder cancer susceptibility in this population.

The first report on miRNA expression profiling in gallbladder cancer was performed in transgenic BK5. erbB2 mice, in which gallbladder cancer was induced by expressing murine ErbB2 gene under bovine keratin 5 promoter in the basal layer of epithelial tissues by Kitamura et al. [59]. MiRNA profiling of 368 miRNAs revealed 9 and 13 significantly upregulated and downregulated miRNAs, respectively, in the gallbladder cancer tissues of these transgenic mice compared with the wide-type mice (>2.2-fold, \( p < 0.05 \)). Furthermore, treatment with histone deacetylase inhibitor PCI-24781 significantly reversed these deregulated miRNAs. For instance, miR-21, miR-142-3p, miR-142-5p, and miR-223, which were upregulated in gallbladder cancer tissue, were decreased upon PCI-24781 treatment. In contrast, miR-122, which was downregulated in gallbladder cancer, was significantly upregulated by PCI-24781, implicating the potential chemotherapeutic value of histone deacetylase inhibition and reversal of miRNA dysregulation in treating biliary tract cancers.

Further studies on miRNAs expression profiling also identified a number of significantly deregulated miRNAs in gallbladder cancer tissues, which are listed in Table 1. For example, Letelier et al. [60] utilized microarray to profile miRNA expression in 6 cancerous and 4 normal gallbladder tissues. Dysregulated miRNAs were further validated by TaqMan RT-PCR in an independent cohort of 8 tumors and 3 non-cancerous gallbladder samples. Through this two-stage approach; the authors confirmed the downregulation of miR-133a, miR-133b, miR-143, miR-145, miR-1, miR-148 and miR-29 c. Pathway enrichment analysis revealed that the most downregulated miRNAs (miR-1, miR-133, miR-143 and miR-145) collectively targeted a number of genes belonging to signaling pathways involved in tumor pathogenesis, such as transforming growth factor (TGF)-β, ErbB3, WNT, vascular endothelial growth factor (VEGF), and those regulating cell motility or adhesion. Functional characterization revealed that miR-1 and miR-145 could significantly inhibit cell viability and induce apoptosis in cultured gallbladder cancer NOZ cells, substantiating their roles as tumor suppressors in gallbladder cancer.

Dicer and Drosha are two key enzymes that are involved in the biogenesis of mature miRNAs [61, 62]. The expression of these two genes was detected in 21 non-dysplastic gallbladder epithelia and 108 gallbladder cancer tissues using immunohistochemical staining [55]. It was demonstrated that Dicer and Drosha expression was significantly lower in gallbladder cancer than that in non-dysplastic gallbladder epithelia. The absence or low expression of Dicer or Drosha was also associated with poor differentiation, lymph node metastasis, invasiveness, and failure of radical resection. Univariate Kaplan–Meier analysis showed that the loss of Dicer and Drosha expression was predictive of decreased overall survival independent of other clinic pathological parameters. Taken together, impairment of miRNA biogenesis contributes to metastasis, invasion, and poor prognosis in gallbladder cancer [63].

A number of deregulated miRNAs have been reported to function as tumor suppressors or oncogenes in gallbladder cancer via derepressing important signaling mediators along specific signaling pathways pertinent to cancer development.

Upregulated MiRNAs in Gallbladder Cancer

The biological functions and/or prognostic
significance of three upregulated miRNAs, namely miR-20a, miR-155 and miR-182, have been studied in details. MiR-20a was up-regulated in gallbladder cancer tissues as demonstrated by both real-time RT-PCR and in situ hybridization. Overexpression of miR-20a promoted invasion and proliferation of gallbladder cancer cells, accompanied by dysregulation of several epithelial–mesenchymal transition-related genes in vitro and in vivo. Smad7 (mothers against decapentaplegic homolog 7), a potential inhibitor of TGF-β1 signaling pathway, was identified as the direct target of miR-20a. Clinically, patients with higher miR-20a levels exhibited worse overall survival [64]. Kono et al. reported that miR-155 was significantly overexpressed in gallbladder cancer when compared with gallbladders with pancreaticobiliary maljunction and normal gallbladders [65]. The high expression level of miR-155 in gallbladder cancer was significantly associated with the presence of lymph node metastasis and poor prognosis. In vitro assays showed that aberrant expression of miR-155 significantly enhanced gallbladder cancer cell proliferation and invasion. Qiu et al. found that miR-182 levels was significantly upregulated in GBC tissues compared with normal controls, and miR-182 expression was remarkably increased in primary tumors that subsequently metastasized, when compared to those non-metastatic tumors [66]. Interestingly, TGF-β induced miR-182 expression in gallbladder cancer cells whereas overexpression of miR-182 promoted gallbladder cell migration and invasion. Importantly, miR-182 inhibition suppressed TGF-β-induced cancer

Table 1: MiRNA expression profiles in GBC

| Num | Method        | sample                  | upregulated                    | downregulated   | Reference |
|-----|---------------|-------------------------|--------------------------------|-----------------|-----------|
| 1   | PCR-RFLP      | primary GBC tissues     | miR-21, miR-142-3p, miR-142-5p, miR-223 | miR-122         | 58        |
| 2   | Microarray RT-PCR | mice                   | miR-133a, miR-133b, miR-143-3p, miR-145-5p, miR-99a-5p, miR-125b-5p, miR-1, miR-29c-3p, miR-195-5p, miR-139-5p, miR-129-5p, miR-100-5p, miR-143-5p, miR-148a-3p, miR-145-3p, miR-376c, miR-187-3p, miR-365a-3p, miR-29b-3p, miR-497-5p, miR-654-3p, miR-411-5p, miR-125a-5p, miR-26a-5p, miR-101-3p, miR-495, miR-381-3p, miR-154-5p, miR-99a-3p, miR-328, miR-299-5p, miR-30e-3p, miR-29b-2-5p, miR-379-5p, miR-140-5p, miR-24-1-5p, miR-101-5p | 59        |
| 3   | Microarray RT-PCR | primary GBC            | miR-133a, miR-133b, miR-143-3p, miR-145-5p, miR-99a-5p, miR-125b-5p, miR-1, miR-29c-3p, miR-195-5p, miR-139-5p, miR-129-5p, miR-100-5p, miR-143-5p, miR-148a-3p, miR-145-3p, miR-376c, miR-187-3p, miR-365a-3p, miR-29b-3p, miR-497-5p, miR-654-3p, miR-411-5p, miR-125a-5p, miR-26a-5p, miR-101-3p, miR-495, miR-381-3p, miR-154-5p, miR-99a-3p, miR-328, miR-299-5p, miR-30e-3p, miR-29b-2-5p, miR-379-5p, miR-140-5p, miR-24-1-5p, miR-101-5p | 60        |
cell migration and invasion. Blockade of miR-182 also effectively inhibited pulmonary metastases in vivo. Cell adhesion molecule 1 (CADM1) was further identified as a new target of miR-182. In this regard, miR-182 inhibited CADM1 expression in vitro and in vivo.

**DOWNREGULATED miRNAs IN GALLBLADDER CANCER**

In stark contrast to the scarcity of reported upregulated miRNAs in gallbladder cancer, miRNA downregulation is more pervasive, suggestive of the tumor-suppressing function of miRNAs as a whole. MiR-335 levels was one of the most significantly underexpressed miRNAs in gallbladder cancer, in which 58% cases exhibited downregulation when compared with their adjacent nondysplastic counterparts as measured by RT-PCR [67]. Clinic pathological correlation further showed that miR-335 expression was significantly lower in gallbladder tissues with high histological grade, advanced pathologic T (tumor) and clinical stages, and lymph node metastasis. Univariate and multivariate analyses further revealed that miR-335 levels could serve as an independent prognostic marker for overall survival. MiR-34a is another hallmark downregulated miRNA in gallbladder cancer. Jin et al. measured miR-34a expression and telomere length in 77 gallbladder cancer tissues and 36 peritumoral tissues [68]. Significant downregulation of miR-34a and longer telomere length were observed in gallbladder cancer tissues, in which such alterations were correlated with poor prognosis. Mechanistically, restored expression of miR-34a inhibited the colony-forming ability of CD44+CD133+ gallbladder cancer stem-like cells in vitro and the growth of tumor xenograft in vivo. Adenovirus-mediated expression of miR-34a could also downregulate PNUTS, a protein that prevents telomere shortening, and reduce telomere length in gallbladder cancer xenograft. Aside from miR-335 and miR-34a, miR-130a was markedly downregulated in gallbladder cancer tissues in which miRNA-130a levels were negatively correlated with HOTAIR, a trans-regulatory long noncoding RNA (lncRNA) [69]. The authors reported that knockdown of HOTAIR inhibited the invasion of gallbladder cancer cells while miRNA-130a inhibitor reversed the decrease in invasiveness. Knockdown of HOTAIR also suppressed cancer cell proliferation as manifested as the reduction

| Name  | Up or down regulation | Target gene | role         | Reference |
|-------|-----------------------|-------------|--------------|-----------|
| miR-20a | Up                    | Smad7       | oncogene     | 64        |
| miR-155 | Up                    |             | oncogene     | 65        |
| miR-335 | Down                  | Tumor suppressor | 66        |
| miR-29b | Up                    |             | oncogene     | 67        |
| miR-200a | Up                    |             | oncogene     | 67        |
| miR-21  | Up                    | PTEN        | oncogene     | 67        |
| miR-34a | Down                  | PNUTS       | Tumor suppressor | 68 |
| miR-130a | Down                  | HOTAIR      | Tumor suppressor | 69 |
| miR-182 | Up                    | CADM1       | oncogene     | 70        |
| miR-26a | Down                  | HMGA2       | Tumor suppressor | 71 |
| miR-135a-5p | Down               | VLDLR       | Tumor suppressor | 72 |
| miRNA-218-5p | Down              | Bmi1        | Tumor suppressor | 73 |

Table 2: Functional characterization of the deregulated miRNAs in GBC
of S-phase fraction while miRNA-130a inhibitor rescued the proliferation. These results implied that the oncogenic effect of HOTAIR was partly mediated through negative regulation of miRNA-130a. Zhou et al. reported that miR-26a [70] and miR-135a-5p [71] levels were significantly reduced in primary gallbladder cancer tissues and their downregulation were associated with poor histological grades. Reintroduction of miR-26a significantly inhibited cell proliferation through induction of G1/S cell cycle arrest. Furthermore, high mobility group AT-hook 2 (HMGA2) was found to be the direct target of miR-26a in gallbladder cancer in which HMGA2 mRNA levels and miR-26a levels were negatively correlated. Similar to miR-26a, re-expression of miRNA-135a-5p inhibited gallbladder cancer cell proliferation in vitro and in vivo, with induction of G1/S cell cycle arrest and upregulation of caspase 3/7 activities. Luciferase reporter assay further demonstrated that miR-135a-5p repressed cell proliferation through direct targeting of very-low-density lipoprotein receptor (VLDLR) and repressed p38 MAPK pathway. Ma et al. [72] reported that overexpression of CCAT1, an IncRNA, in gallbladder cancer contributed to upregulation of Bmi1, which is the target of miRNA-218-5p. Subsequent analysis confirmed that CCAT1 decreased the availability of miRNA-218-5p by functioning as a ‘miRNA sponge’. These data revealed that CCAT1 enhanced the proliferation and invasiveness of gallbladder cancer cells, at least in part, through disrupting miRNA-218-5p-mediated downregulation of Bmi1. Moreover, CCAT1 transcript levels were correlated with that of Bmi1 in gallbladder cancer tissues. Aquaporins (ACQs) are important in controlling bile formation and could exert oncogenic action if overexpressed in gallbladder cancer. AQP-5 silencing by siRNA restored the expression of miR-29b, -200a, and -21 in gallbladder cancer cells, suggesting the downregulation of these miRNAs might mediate the oncogenic action of ACQs [73] (Table 2).

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Increasing evidence has confirmed the importance of miRNA dysregulation in the progression and pathogenesis of human malignancies including gallbladder cancer. The functional roles of specific miRNAs as oncogenes or tumor suppressors render them attractive targets for therapeutic intervention. Nevertheless, with more research efforts put forth to the development of miRNA-based therapeutics and delivery system, it is hopeful that miRNAs will achieve clinical utility at last.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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