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Review Article

The Multiple Molecular Signatures in Gallbladder Carcinoma: from Basic Studies to Clinical Application

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Abstract

Gallbladder carcinoma (GBC) is a malignant tumor in gastrointestinal system. In this review, we mainly focus on three molecular levels to illuminate the potential molecular mechanisms in gallbladder carcinoma. First, we review genes with mutation and methylation associated with occurrence and development of GBC. Second, we review non-coding RNA and key differential genes at the transcript level, especially for their interactions. Third, we review crucial proteins in GBC. Moreover, we also discuss the challenges of these molecular signatures in clinical applications. Finally, we discuss potential application of these crucial genes in prevention, diagnosis and treatment of GBC.

Keywords: Gallbladder carcinoma (GBC); Diagnosis; Treatment

Abbreviations: mRNA-messenger RNA; miRNA-microRNA; ncRNA-non-coding RNA; lncRNA-long non-coding RNA; GBC-gallbladder carcinoma

1. Introduction

Gallbladder carcinoma (GBC) is the most common biliary tract malignancy and has a poor prognosis, frequently presenting at an advanced stage. Prevalence rates of up to 7.5 per 100,000 for men and 23 per 100,000 for women have been reported from Andean-area populations, North-American Indians, Mexican Americans and inhabitants of Northern India [1]. Fewer than 10% of GBC patients survive more than 5 years after treatment [2]. GBC is hard to detect in the early stage, and most patients cannot be cured by surgery after detection because of the invasion of cancerous cells. For dozens of years of clinical practice, surgery was the only effective way to treat GBC. However, surgery usually cannot cure patients because the pathogeny of GBC has not been completely disclosed and the invasive cancerous cells cannot be eliminated surgically. Therefore, novel methods may be important ways to cure GBC, such as targeted therapy, which has broad application [3].

In this review, we focus on the detection and treatment signatures of GBC with the goal of illustrating the crosstalk among DNA, RNA and protein levels (Figure 1), and we expect that this review will provide useful information for the clinical diagnosis and treatment of GBC.

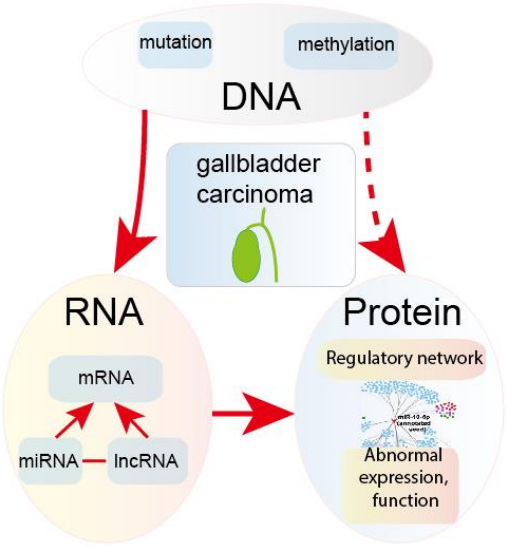

Figure 1: The three molecular signature levels are reviewed.

2. Genomic Signatures of GBC

Here, the molecular signatures at DNA level mainly include mutation and methylation. The mutation of coding genes provides great advantages in the diagnosis and guidance of treatment. Research in Chile showed that allele-specific mutations could affect the incidence of GBC [4]. Inherited rare germline mutations had been proved to be related to GBC [5] and epidermal growth factor receptor mutations may have advantages for the treatment of gallbladder cancer [6]. Furthermore, some key genes, like P53, K-ras, Keap1, PIK3CA, EGFR, P16 and B-raf, have

been explored. For example, P53 mutation in exons 5-8 were found in GBC patients and K-ras in codon 12 was important in the early stage [7, 8]. Mutation of Keap1 was found in C249Y and S338L led to the loss of Nrf2 repression activity [9]. Moreover, PIK3CA mutation in exons 9 was found specifically and may be effective in targeted therapies [10]. EGFR mutation in exons 19-21 could sustain survival and proliferation of GBC cells [11] and high percentage of B-raf mutations in exon 15 was found [12]. Therefore, gene mutation in GBC is meaningful for the understanding and treatment of GBC (Table 1).

The methylation of DNA also greatly contributes to diagnosis and therapy. A recent study showed that the patterns of gene promoter methylation allowed them to be considered biomarkers for the early detection, diagnosis, prognosis and therapeutic selection [13]. Furthermore, gene-specific DNA methylation (such as APC, CDKN2A, ESR1, PGP9.5 and SSBP2) had the same function [14]. Another study in north-central India proved D4Z4 and DNF92 subtelomeric sequences to be hypermethylated and hypomethylated, respectively [15]. Aberrant hypermethylation of promoter regions was an early, progressive and cumulative event in GBC [16]. One study on the methylation profile of GBC showed that the methylation profile was different from that in a healthy individual, which proved that methylation was an early event [17]. MYC hypomethylation was only detected in tumoral samples and was associated with its protein expression (p=0.029) and MYC mutations were detected in 80% of GBC samples [18].

Genes	Mutation position	Ref.
p53	exons 5,6,7,8	[7]
k-ras	codon 12 (GGT change to GAT)	[8]
Keap1	C249Y/S338L	[9]
PIK3CA	exons 9	[10]
EGFR	exons19,20,21	[11]
P16	exons 1, 2	[8]
B-raf	exon 15	[12]

Table 1: The study of some key mutation genes in GBC.

3. Crucial mRNAs and ncRNAs in GBC

The most effective way to cure GBC is to diagnose the cancer in the early stage, but the disappointing reality is that GBC remains difficult to diagnose preoperatively. Furthermore, extension of the disease beyond the mucosa predicts a poor chance of long-term survival [19]. Increasing studies have shown that miRNAs, mRNAs and long non-coding RNAs are very effective in the diagnosis of GBC. mRNAs in GBC greatly affect physiological and pathophysiological conditions. Among these, human telomerase reverse transcriptase (hTERT) mRNA, a catalytic subunit of telomerase, had been determined to be effective for diagnosing the nature of the polypoid lesion in the gallbladder [20], which can help to diagnose GBC. Survivin, an inhibitor of apoptosis, played a possible role and

was associated with poor prognosis [21]. SUMO-1 mRNA may be an interesting target in the diagnosis and treatment of GBC based on the differences expression in carcinoma of gallbladder, gallbladder tissues surrounding GBC, adenomatous polyp of gallbladder [22]. In addition, research on the positive expression of CD-146 [23], VEGF, Flt-1 and KDR [24] were also related to the incidence of GBC.

miRNA is involved in the initiation and progression of GBC (Table 2), and miRNA expression profiling can be used to identify signatures associated with diagnosis, staging, progression, prognosis and response to treatment [25]. miRNAs participate in a variety of regulatory pathways, and the expression level of miRNAs is closely connected to survival of patients with GBC. For example, patients with a high miRNA-155 expression level had distinctly shorter overall survival than patients with a low miRNA-155 expression level [26]. In addition, high miR-155 expression was related to aggressive GBC, and it may be a potential prognostic marker and therapeutic target [27]. miR-146b-5p inhibited growth of GBC by targeting epidermal growth factor receptor [28]. miR-335 may be associated with aggressive tumor behaviors and reduced expression of miR-335 may be a useful indicator for clinical outcome and could be a therapeutic target for primary GBC [29]. miR-29c-5p, a tumor-suppressive miRNA that may serve as a potential prognostic biomarker or therapeutic target for GBC, suppressed GBC progression by directly targeting CPEB4 and inhibiting the MAPK pathway [30]. Moreover, miR-133a-3p acted as a tumor suppressor by directly targeting the recombination signal-binding protein Jk (RBPJ) in GBC [31]. Another miRNA, miR-26a, may contribute to GBC proliferation by directly targeting HMGA2 and might be a prognostic factor and therapeutic target for GBC patients [32].

miRNA	**Expression(miRNA)**	**mRNA**	**Expression(mRNA)**	**Ref.**
miR-146b-5p	down	EGFR	up	[28]
miR-20a	up	Smad7	down	[44]
miR-29c-5p	down	CPEB4	up	[30]
miR-133a-3p	down	RBPJ	up	[31]
miR-26a	down	HMGA2	up	[32]
miR-122	down	PKM2	up	[45]
miR-143-3p	down	ITGA6	up	[46]
miR-101	down	ZFX	up	[47]
miR-30d-5p	down	LDHA	up	[48]
miR-182	up	CADM1	down	[49]
miR-30b	down	NT5E	up	[50]
miR-340	down	NT5E	up	[50]
miR-125b-5p	down	Bcl2	up	[51]
miR-143-5p	down	HIF-1α	up	[52]
miR-33a	down	IL-6	up	[53]

miR-223	down	STMN1	up	[54]
miR-218-5p	down	PRKCE	up	[55]

Table 2: Some abnormal miRNAs in GBC and their target mRNAs.

Through recent research, long non-coding RNAs also have been determined to play important roles in GBC pathogenesis [33]. Long non-coding RNA SPRY4-IT1 promoted GBC progression and may serve as a candidate target for new therapies in human GBC [34]. In addition, CRNDE was found an important contributor to GBC development as a scaffold to recruit the DMBT1 and c-IAP1 and affect PI3K-AKT pathway [35]. Furthermore, upregulation of HOXA-AS2 promoted proliferation and induced epithelial-mesenchymal transition in GBC, and it could be as a potential therapeutic target to inhibit GBC metastasis [36]. Another study showed that UCA1 promoted GBC cell proliferation and metastasis in vitro and suppressed the transcription of p21 and E-cadherin by recruiting an enhancer of the zeste homolog [37]. Moreover, lncRNA-H19 was a novel prognostic factor for GBC and might play important regulatory roles in the epithelial-mesenchymal transition (EMT) process [38]. Overexpression of lncRNA-LET conferred a proliferative advantage to tumor cells under hypoxic conditions. The ectopic expression of lncRNA-LET led to promotion of cell cycle arrest at G0/G1 phase and induction of apoptosis under hypoxic conditions. Abnormal expression of lncRNA-LET also suppressed gallbladder tumor growth, and lncRNA-LET was determined to a potential prognostic marker and therapeutic target for GBC [39].

Indeed, long non-coding RNAs have important interactions with mRNAs and miRNAs (Table 3 and Figure 2). For example, H19 regulated FOXM1 expression by competitively binding endogenous miR-342-3p [40] and GCASPC and miR-17-3p interaction regulated cells proliferation [41]. LINC00152 negatively regulated Bag-1 as a molecular sponge for miR-138 [42] and CCAT1 negatively regulated Bmil by spongeing miR-218-5p [43].

LncRNA	**Expression (LncRNA)**	**miRNA**	**Expression (miRNA)**	**mRNA**	**Expression (mRNA)**	**Ref.**
LINC00152	up	miR-138	down	HIF-1a	up	[42]
CCAT1	up	miR-218-5p	down	Bmi1	up	[43]
HOTAIR	up	miR-130a	down	c-Myc	up	[56]
H19	up	miR-194-5p	down	AKT2	up	[57]
H19	up	miR-342-3p	down	FOXM1	up	[40]
MALAT1	up	miR-363-3p	down	MCL-1	up	[58]
MALAT1	up	miR-206	down	KRAS	up	[59]
MALAT1	up	miR-206	down	ANXA2	up	[59]
PAGBC	up	miR-511	down	PIK3R3	up	[60]
PAGBC	up	miR-133b	down	SOX4	up	[60]

TUG1	up	miR-300	down	TGF-β1	up	[61]
MINCR	up	miR-26a-5p	down	EZH2	up	[62]
GCASPC	down	miR-17-3p	up	pyruvate carboxylase	up	[41]

Table 3: Some examples of the relationship between lncRNAs and micRNAs/mRNAs in GBC.

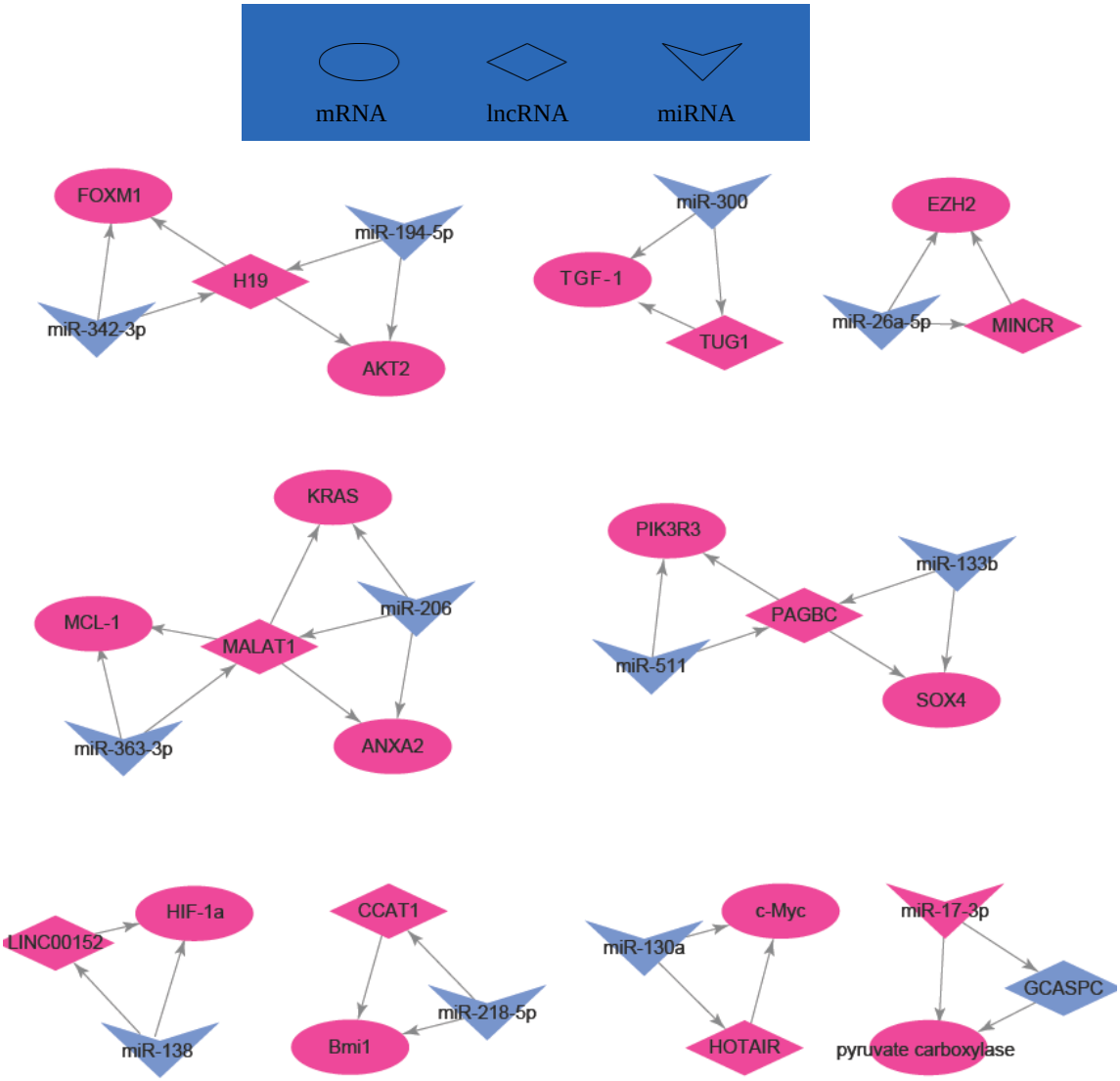

Figure 2: Regulation network of some lncRNAs, mRNAs and miRNAs in GBC.

4. Crucial proteins in GBC

Compared with molecules above mentioned, crucial proteins also contribute to diagnosis and treatment of GBC [65, 72]. The modification of proteins, mainly including protein phosphorylation and acetylation, may contribute to abnormal expression and/or function. Many proteins impacting on GBC have been reported (Table 4).

Protein	Expression	Tissue	Ref.
Bcl-2	up	gallbladder tissue	[63]
c-erb-B2	up	gallbladder tissue	[64]
MMP-2	up	gallbladder tissue	[65]
TIMP-2	down	gallbladder tissue	[65]
VEGF	up	gallbladder tissue specimens (formalin-fixed and paraffin-embedded)	[66]
FHIT	down	gallbladder tissue	[67]
MLH1	down	gallbladder tissue	[67]
retinoblastoma	up	gallbladder tissue	[68]
p16INK4	down	gallbladder tissue	[68]
S100A4	up	gallbladder tissue	[69]
P53	up	gallbladder tissue	[69]
p27	down	gallbladder tissue	[69]
P16	down	gallbladder tissue	[69]
RB	down	gallbladder tissue	[69]
Smad4	down	gallbladder tissue	[69]
FHIT	down	gallbladder tissue	[69]
E-cadherin	down	gallbladder tissue	[69]
promyelocytic leukemia (PML)	down	gallbladder tissue	[69]
CDX2	up	GBC cell lines	[70]
cyclin D1	up	gallbladder tissue specimens (formalin-fixed and paraffin-embedded)	[71]
p16	down	gallbladder tissue specimens (formalin-fixed and paraffin-embedded)	[71]
retinoblastoma	up	gallbladder tissue specimens (formalin-fixed and paraffin-embedded	[71]
MUC1 mucins	up	gallbladder tissue	[72]
MUC4	up	gallbladder tissue	[73]

CD133	up	gallbladder tissue	[74]
transcription factor specificity protein 1 (SP1)	up	gallbladder tissue/GBC-SD cell lines	[75]

Table 4: Some key proteins in GBC.

For example, the overexpression of Bcl-2 could promote tumor cell differentiation [63]. The overexpression of c-erb-B2 was related to the worse prognosis of GBC [64] and the ratio of MMP-2/TIMP-2 may be a new significant marker in early diagnosis [65]. Reduced Fhit expression might be involved in the development of GBC and be correlated with Mlh1 expression [67]. The high expression of retinoblastoma protein inhibited P16INK4 protein and related to the decreased survival in GBCs [68]. Furthermore, the overexpression of S100A4, P53 and the loss of p27, p16, RB, Smad4, FHIT, E-cadherin and PML expression led to poor survival. PML and P53 were found effective therapeutic targets for the disease [69]. The research on GBC cell lines showed that the overexpression of SP1 [75] and CDX2 appeared in most GBC cell lines, and the expression of CDX2 showed a relationship with the expression of MUC2 [70]. A study in gallbladder tissue specimens (formalin-fixed and paraffin-embedded) showed that cyclin D1 had a negative-correlation with P16 and affected the early stage of GBCs [71]. As mentioned above, the expression of protein may correlate with each other and affect disease diagnosis.

Phosphorylation also contributed to diagnosis and treatment of GBC [76]. For example, CD133 had a role in the migration of GBC cells through phosphorylation [74] and MUC4 interacted with ErbB2 to cause tumor growth, which was related to the hyperphosphorylation of ErbB2, MAPK and Akt [73]. Research on cirsimaritin showed that the pro-apoptotic effect of cirsimaritin could be reversed by down-regulating the phosphorylation of Akt [77] and affected the GBC-SD cell line. In addition, the protein phosphatase PHLPP was found to help treat GBC by inhibiting survivin phosphorylation [78]. Rise of CCK1 receptor expression is associated with the increase of protein lysine acetylation [79], showing that GBC can be treated by using histone deacetylase inhibitor [80].

5. Challenges and Conclusion

Although many factors are related to GBC, but GBC still cannot be diagnosed accurately in clinical settings. A clinical experiment in the Queen Mary Hospital, University of Hong Kong showed that 61% of patients had an inaccurate diagnosis [81], which showed that diagnosis is difficult in the early stage. Operative resection is still the only way to cure GBC, but unfortunately, only 38% of patients have been eligible for resection during the last 20 years. Furthermore, one report showed that none of the patients without surgery survived more than 5 years [82]. Therefore, the challenges of treating GBC are serious because only surgery in the early stage can cure GBC, but most patients are diagnosed at the intermediate or advanced stage.

Because of the current challenges and problems in the diagnosis and treatment of GBC, new methods for treating GBC must be developed. To this end, targeted therapy may be an effective way to cure GBC. Targeted therapy has

been proved to affect GBC in clinical trials in the Clinical Center for Targeted Therapy at the MD Anderson Cancer Center [83]. GBC still cannot be readily cured by targeted therapy, especially in the advanced stage, and almost all patients who do not have surgery currently die within 1 year following clinical treatment [84]. Chemotherapy is another adjuvant therapy for GBC in clinical settings. During a randomized controlled study, patients who underwent chemotherapy after surgery had a higher 5-year survival rate than those with surgery alone. However, adverse drug reactions, such as anorexia and leukopenia, were usually associated with chemotherapy [85].

Research on the pathogenesis and prognosis of GBC has made great progress, and genes have been determined to be possible markers for prognosis. Lupeol in the EGFR/MMP-9 pathway was proved to induce apoptotic cell death in GBC, which can be used as a potential treatment method [86]. Long non-coding RNAs of LINC00152 and CRNDE in the PI3K-AKT pathway contribute to process of GBC carcinogenesis, and PI3K/AKT may be a potential therapeutic target for GBC [75, 85].

Taken together, in this review, the multiple molecular signatures in GBC were concluded. We hope that these potential signatures can provide some references for diagnosis and therapeutic of GBC.

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Conflict of Interest

None

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