Meta-signature of mutated genes in gallbladder cancer: evidence based high throughput screening assays

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Background

Gallbladder carcinoma (GBC) is the fifth most common carcinoma of gastrointestinal tract, and represents 80–95% of biliary tract cancers. It is relatively an uncommon malignant disease with a poor prognosis. According to previous reports (1), GBC has a low incidence rate (<2/100,000). Reid *et al*. (2) found that the worldwide incidence of GBC correlates with the prevalence of gallstone disease. The high-incidence areas of GBC are Poland (14/100,000), Northern India (21.5/100,000), south Pakistan (11.3/100,000), Israel (5/100,000) and Japan (7/100,000) (1). Besides, GBC is more common in females. Stinton *et al*. (1) demonstrated that the incidence rate was high in South American females, 15.5 per 100,000 in Bolivia (*vs*. 7.5/100,000 in male), and 11.3 per 100,000 in New Mexico (*vs*. 4/100,000 in male).

A satisfied outcome depends on the early diagnosis and appropriative treatment. Up to date, the most effective treatment for GBC patients is surgery. However, mainly due to their occult symptoms, less than 10% of GBC patients have the opportunities to receive surgery, and nearly 50% of them already had lymph node metastasis at first diagnosis. Because of the difficulties in early diagnosis, the prognosis of GBC is so poor. The overall 5-year survival rate of GBC patients is less than 5% (3). A thorough understanding of the underlying mechanism is critical for exploring potential diagnostic biomarker and developing effective therapeutic approach for GBC patients.

## High-throughput genetic mutation profiling in GBC

Grateful thanks to the decades of relevant studies, a numerous molecular mechanisms involved in GBC were unveiled. Recently, molecular testing in multiple solid tumors has become standard practice. Newer molecular tests are focusing on mutation detection as a diagnostic biomarker of GBC. High-throughput genetic mutation profiling provided the possibility to do the comprehensive examination of the cancer genome. It has undoubted advances in the characterization and quantification of genomes, epigenomes and transcriptomes. High-throughput genetic mutation profiling is being widely applied in mutation detection. Today, several commercial platforms are available, including SNaPshot multiplex system, next generation sequencing (NGS) and massARRAY platform technics. Among of them, NGS technology is widely applied high-throughput genetic mutation detection method since 2006. NGS technology is free from many of the confines dictated by previous technologies, such as the bias due to the probe selection in array technology, cross-hybridization background, and signal saturation-induced detection dynamic range limitation.

Recently, Javle *et al*. (4) performed mass spectroscopy-based and next-generation sequencing profiling in GBC samples. By hotspot mutations analysis, they found 14 hotspot mutations from 11 different genes, included IDH1, KRAS, NRAS, PIK3CA and MET. Among of them, mutations in IDH1 are the most recurrent (36.4%). They

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Table 1 Characteristics of analyzed datasets

Author (year)	Country	Ethnicity	No. of patients	Sample type	Assay platform	Refs.
Borger *et al*. [2012]	USA	Caucasion	25	FFPE	SNaPshot multiplex system	(5)
Jiao *et al*. [2013]	USA	Caucasion	9	Fresh-frozen	Whole-exome sequencing	(6)
Javle *et al*. [2014]	USA	Caucasion	72	FFPE	Targeted sequencing	(4)
Kumari *et al*. [2014]	India	Asian	49	FFPE	MassARRAY platform	(7)
Simbolo *et al*. [2014]	Italy	Caucasion	26	FFPE	Targeted sequencing	(8)
Li *et al*. [2014]	China	Asian	51	Fresh-frozen	Whole-exome sequencing	(9)

FFPE, formalin-fixed paraffin-embedded.
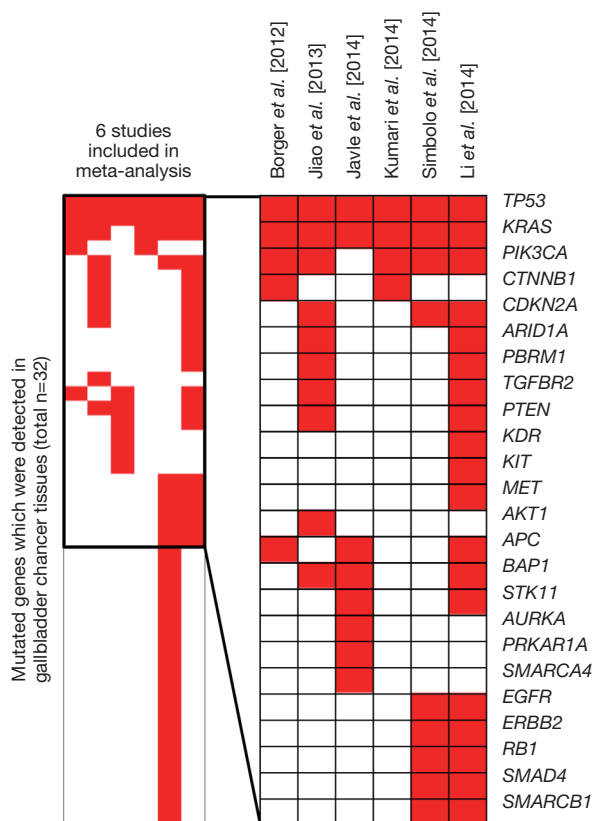
Figure 1 Meta-signature of mutated genes in gallbladder carcinoma (GBC).

Table 2 Meta-signature mutations in gallbladder cancer

Genes	Studies	P value	Corrected P value
TP53	5	1.44×10^{-6}	1.00×10^{-3}
KRAS	5	3.37×10^{-6}	2.34×10^{-2}
PIK3CA	4	1.10×10^{-4}	7.65×10^{-2}

also detected 26 mutations by targeted NGS, and identified TP53 as the most common mutated gene. They further conducted a multivariate analysis and found mutated IDH and KRAS were associated with poorer overall survival. Their results provided evidence that high-throughput mutation profiling may be a useful platform for identifying novel mutations for targeted therapy of GBC.

Meta-signature of mutated genes in GBC

Nowadays, increasing groups are focusing on mutated genes in GBC. However, due to small sample size and different technological platforms between above studies, the mutated gene profiling effort in GBC led to inconsistent results. To overcome the limitations, we conducted a meta-signature of mutated genes in GBC based on six studies (4-9) including 232 subjects receiving high-throughput genetic mutation profiling (*Table 1*). Totally 43 mutated genes were detected in 232 GBC patients. Among of them, six genes (TP53, KRAS, PIK3CA, CDKN2A, BAP1 and APC) were reported in more than three studies (*Figure 1*). Our meta-analysis further revealed that three mutated genes (TP53, KRAS, PIK3CA) were significantly associated with GBC (*Table 2*). In the following aspect, we will discuss the three recurrent mutated genes.

TP53 contains 34,453 mutations, including 1,311 hotspot mutations (10). Increasing evidence suggest that mutated TP53 plays important role in multiple tumors. Cardesa *et al*. (11) represented that TP53 gene mutations were observed in up to 50% of head and neck squamous-cell carcinomas and approximately 65% of them have aberrant expression of TP53. Szymańska *et al*. (12) also reported that TP53 was the most frequently mutated gene in human cancer, such as hepatocellular carcinoma and oesophagus

carcinoma. Asai *et al*. (13), exploredTP53 mutations in GBC patients, and found nearly half of GBC patients have TP53 mutations. In our meta-analysis, we also found that TP53 was the most recurrent mutated gene in GBC (crude P value =1.44×10^{-6}, corrected P value =1.00×10^{-3}, *Table 2*).

There are more than 3,000 in KRAS, and 90% of them are located in exon 2 and 10% in exons 3 and 4 (www.sanger.ac.uk/genetics/CGP/cosmic/). KRAS has been considered as one of the most frequently mutated genes in multiple tumors. Therkildsen *et al*. (14) meta-analyzed 22 studies with 2,395 patients with different tumors, and found that KRAS mutations might be implemented for prediction of clinical benefit from anti-EGFR antibodies in metastatic colorectal cancer. Eirini *et al*. (15) explored KRAS mutations in non-small-cell lung cancer patients, and represented that KRAS exon 2 mutation was observed in 18.89% (106/561) patients. Reid *et al*. (2) reported that KRAS mutations were associated with GBC in patients with anomalous junction of the pancreaticobiliary duct (AJPBD), suggesting that KRAS mutation might serve as a useful tool in screening early GBC in patients with AJPBD. Our data also revealed that mutated KRAS was associated with GBC (crude P value =3.37×10^{-6}, corrected P value =2.34×10^{-2}, *Table 2*), consistent with previous studies.

PIK3CA is located on 3q26.3, whose mutations were also associated with multiple malignancies. Dey *et al*. (16) found that PIK3CA mutations were detected in 35% patients with breast cancer, which were associated with deregulation of PI3K pathway and contributed to carcinogenesis of breast cancer. Yip *et al*. (17) also reported the relationship between mutated PIK3CA and nasopharyngeal carcinoma (NPC). They performed qRT-PCR and immunohistochemical staining in 74 patients with NPC, and demonstrated that aberrant expression of PIK3CA was detected in 68.9% (51/74) patients with NPC. In GBC, Deshpande *et al*. (18) found PIK3CA mutations in 12.5% patients and suggested PIK3CA mutations as diagnostic biomarkers and therapy targets. In the present study, we also found that mutated PIK3CA was associated with GBC, although the corrected P-value was not significant mainly due to small number of studies (crude P value =1.10×10^{-4}, corrected P value =7.65×10^{-2}, *Table 2*).

Summary and prospect

Overall, our meta-analysis data strongly suggested that mutated TP53, KRAS, PIK3CA were associated with GBC, and it may be a potential diagnostic and prognostic biomarker for GBC patients. However, nowadays, the limited number of studies cannot supply sufficient evidence for further analysis. Therefore, large, multi-center and well-performed studies are warranted to confirm above findings. In future, GBC patients harboring mutations of TP53, KRAS, PIK3CA may benefit from target therapies available or in development.

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Footnote

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