Detection of genetic and epigenetic DNA markers in urine for the early detection of primary and recurrent hepatocellular carcinoma

Hie-Won Hann  
*Thomas Jefferson University Hospital*

Surbhi Jain  
*JBS Science Inc.*

Ting-Tsung Chang  
*National Cheng Kung University Medical Center*

Chi-Tan Hu  
*Buddhist Tzu Chi General Hospital and Tzu Chi University*

Selena Lin  
*Drexel University, College of Medicine*

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Authors
Hie-Won Hann, Surbhi Jain, Ting-Tsung Chang, Chi-Tan Hu, Selena Lin, Wei Song, and Ying-Hsiu Su

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Detection of genetic and epigenetic DNA markers in urine for the early detection of primary and recurrent hepatocellular carcinoma

Hie-Won Hann,1, Surbhi Jain,2, Soojin Yi,3, Ting-Tsong Chang3,4, Chi-Tan Hu,4, Selena Lin,5, Wei Song,5, and Ying-Hsiu Su6

1Thomas Jefferson University Hospital, 2JBS Science, Inc, 3National Cheng Kung University Medical College, 4Buddhist Tzu Chi General Hospital and Tzu Chi University, 5Drexel University, College of Medicine, and 6TheBaruch S. Blumberg Institute

Introduction
Hepatocellular carcinoma (HCC) or liver cancer is an aggressive disease and one of the fastest growing cancers by incidence in the United States. Early detection is the key for effective treatment as the 5-year survival rate is 20% in early stage HCC as compared to only 2% when found after spreading to distant organs. The current marker, alpha-fetoprotein (AFP) and its fucosylated glycoprotein, AFL, are of limited use due to low sensitivity.

Objective
Develop a urine test using a panel of genetic and epigenetic markers for the early detection of primary and recurrent HCC.

Results
Case 1: Detection of a primary HCC

Figure 1: Urine DNA and liver tumor DNA

Microscopic ablation

Case 2: Detection of recurrent HCC

Figure 3: Retrospective analysis of urinary DNA biomarkers plus serum AFP collected during doctor visits of a 55 year old male with HBV-cirrhosis who developed HCC that was undetectable by serum AFP and was treated with microwave ablation. Urinary mRASSF1A DNA and TERT 124 mutated DNA levels started to elevate 8 months prior to HCC detection by MRI and declined post treatment. TP53 249T mutation level rose with the appearance of HCC and declined post treatment.

Case 3: Detection of recurrent HCC

Figure 4: Retrospective analysis of urinary DNA biomarkers and serum AFP levels collected during doctor visits of a 53 year old male with HBV-cirrhosis and HCC that was treated with TACE. Samples were collected before TACE at the first time point. The DNA markers were undetectable but serum AFP was slightly elevated at 86 ng/ml and declined to baseline after TACE therapy. HCC recurrence was detected by MRI 11-13 months after TACE treatment. TERT 124 mutation levels were elevated 6 months prior to recurrence detection by MRI. mRASSF1A, TP53 249T mutation, and serum AFP levels rise with the appearance of recurrent nodule and declined post treatment. This recurrence was also treated with TACE. Note, MRI at 11 months was not definitive. Urine mGSTP1, TERT 124 levels rise 4 months post repeat TACE concurrent with the appearance of a LIRADS category 3 lesion on MRI.

Case 4: Detection of recurrent HCC

Figure 5: Retrospective analysis of urinary DNA biomarkers plus serum AFP collected during doctor visits of a 53 year old male with HBV-cirrhosis who developed HCC that was detected by serum AFP and was treated with microwave ablation. The patient, however, had a recurrence of HCC 4 months post treatment. While mGSTP1 and CTNNB1 mutation levels in urine declined post treatment, urinary mRASSF1A and TERT 124 mutation levels continued to rise concurrent with the recurrence.

Methods
Urine samples were collected with written informed consent and institutional review board approvals from National Cheng Kung University (Tainan, Taiwan), Tzu-Chi Hospital (Hualien, Taiwan), and Thomas Jefferson University Hospital (Philadelphia, PA), at visits with a hepatologist. The samples from Tzu Chi were barred for disease status as a blinded study. Total urine DNA was extracted (Su, Wang et al. 2018) and this molecular weight urine DNA. DNA was then 1:10, was obtained from total urine DNA using cetuximab-bonded magnetic beads (Agencourt Bioscience Corporation) as previously developed by Su et al. (Su, Wang et al. 2018). Baseline (BS) treatment was performed using EZ DNA Methylation-Kit (Zymo Research) for DNA affinity purification to isolate DNA in urine from DNA in urine, we have developed short amplicons (~300 bp) PCRamplified assays for mutations in TP53 (249T) (Lin, Dhillon et al. 2011), CTNNB1 (Lin, Dhillon et al. 2011), TERT (Lin, Dhillon et al. 2011) and for aberrant DNA methylation in GSTP1 (mGSTP1) and RASSF1A (mRASSF1A) (Jain, Xie et al. 2015).

Table 1: Frequent genetic mutations (G) and epigenetic aberrant DNA methylations (M) reported in HCC tissues.

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Gene</th>
<th>DNA methylation</th>
<th>Incidence</th>
<th>Mutation position</th>
<th>Specificity</th>
<th>Sensitivity</th>
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<tr>
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<tr>
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<td>G</td>
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<td>~90%</td>
</tr>
<tr>
<td>CTNNB1</td>
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<td>G</td>
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<td>Yes</td>
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<td>~90%</td>
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<tr>
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<td>Yes</td>
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<tr>
<td>TP53</td>
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<tr>
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<td>G</td>
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<td>~90%</td>
</tr>
</tbody>
</table>

Conclusions

> A total of 10 cases with treated HCC were monitored by serum AFP and urine DNA biomarkers in a blinded study. Of the 10, 4 developed recurrence during the study. HCC-specific urine DNA markers were detected in 3 of 4 patients six months prior to MRI diagnosis and in one patient concurrent with MRI diagnosis.

> Fifteen cirrhosis and nine hepatitis patients were monitored every six months for HCC. In this group, one patient developed primary HCC and the urine DNA biomarkers were detected six months prior to MRI diagnosis.

> The urine test could lead to a paradigm shift for screening and effective management of primary and recurrent HCC and for personalized disease management.

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