DNA Methylation Biomarkers for Noninvasive Detection of Hepatocellular Carcinoma

David Taggart[1], Dhruvajyoti Roy[2], Gen Li[3], Dan Liu[3], Lianghong Zheng[3], and Kang Zhang[4]

[1]Laboratory for Advanced Medicine, Inc., West Lafayette, IN, USA; [2]Laboratory for Advanced Medicine, Inc., Irvine, CA, USA
[3]Laboratory for Advanced Medicine, Inc., Beijing, China; [4]Institute for Genomic Medicine, University of California at San Diego, La Jolla, CA, USA

Abstract

Background: The epigenetic inactivation of tumor suppressor genes by promoter hypermethylation is an important aspect of tumorigenesis. Indeed, aberrant methylation of CpG sites within genomic DNA isolated from cancer cells has been shown to correlate with clinically relevant information and has the potential to be used for cancer diagnosis and identification of the cancer tissue of origin. Malignant cells shed genomic DNA into circulation through both cell death and active release by viable cells. Therefore, investigating the methylation of cell-free DNA allows for the noninvasive detection and early diagnosis of cancers, such as hepatocellular carcinoma (HCC). Here, we identified and validated hepatocellular carcinoma-specific methylation markers for diagnosis of the disease with high sensitivity and specificity.

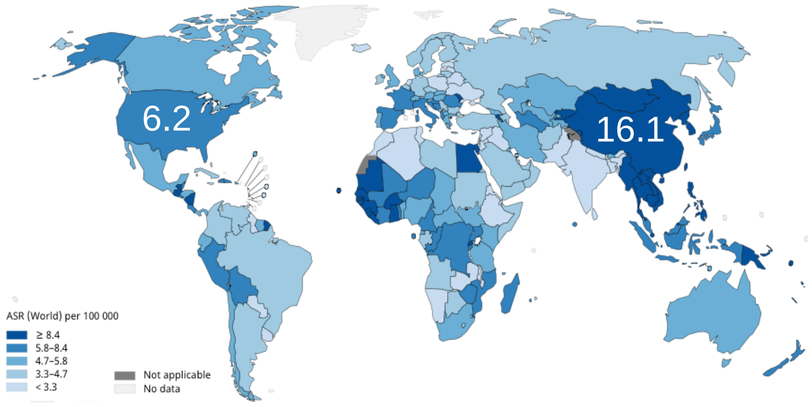
Methods: Banked samples were obtained for 130 subjects, including: 60 subjects diagnosed with hepatocellular carcinoma (Stage I to IV), 30 subjects without liver disease, 10 subjects diagnosed with benign liver disease and 30 subjects diagnosed with breast, colorectal or lung cancer. Samples were provided to the laboratory blinded for analysis. Cell-free DNA was then extracted from the samples, bisulfite converted, and DNA methylation was quantified by using the IvyGene® Platform. After data collection and analysis of all samples was complete, the samples were unblinded to calculate test performance.

Results: A total of 57 of the 60 samples drawn from subjects with hepatocellular carcinoma were correctly identified for an overall calculated sensitivity of 95%, with little difference between the sensitivity of detecting Stage I to Stage IV hepatocellular carcinoma (range 89% to 100%). Additionally, 29 of 30 samples drawn from subjects without liver disease and 10 of 10 samples drawn from subjects diagnosed with benign liver disease were correctly identified as non-cancer for a combined calculated specificity of 97.5%. Of the samples drawn from subjects with cancer other than liver cancer, 90% of breast cancer samples, 80% of colorectal cancer samples, and 90% of lung cancer samples were correctly identified as non-liver cancer, for a total calculated analytical specificity of 87%.

Conclusion: These data demonstrate the high diagnostic potential of cfDNA methylation markers in the blood for the detection of hepatocellular carcinoma. Indeed, quantification of cfDNA methylation may be a more sensitive and specific method for the detection of hepatocellular carcinoma than ultrasound, which is the current recommended imaging method for surveillance of high-risk populations.

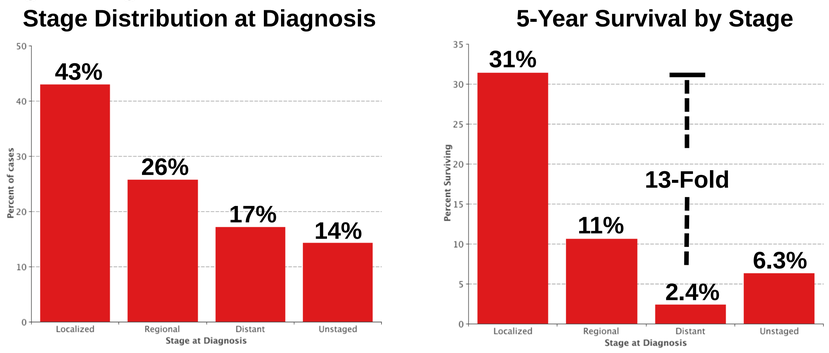
Introduction

Fig 1. WHO Estimated Incidence of Liver cancer in 2018



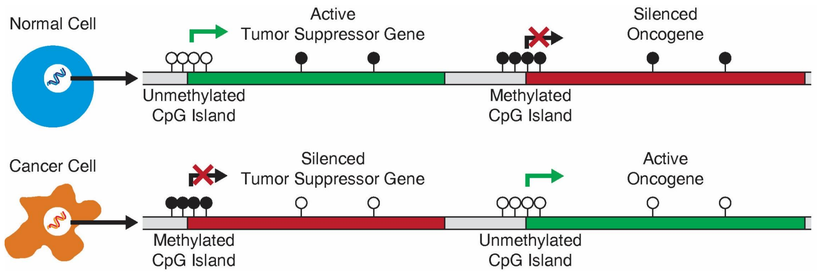
- Incidence of liver cancer is 6.2 per 100,000 in US[1]
- Incidence in China is 2.6-fold higher that the United States due to higher rates of HBV and HCV infection
- An estimated 80% of liver cancers occur in a background of cirrhosis
- Approximately 75% of liver cancers are hepatocellular carcinoma (HCC)
- Approximately 20% are bile duct cancers (Cholangiocarcinoma)

Fig 2. Stage Distribution and Survival of Liver Cancers in US



- Less than half of liver cancers are detected at an early, localized stage
- 5-year survival decreases up to 13-fold if diagnosed at a later stage
- Data includes liver and bile duct cancers, all races, all ages[2]

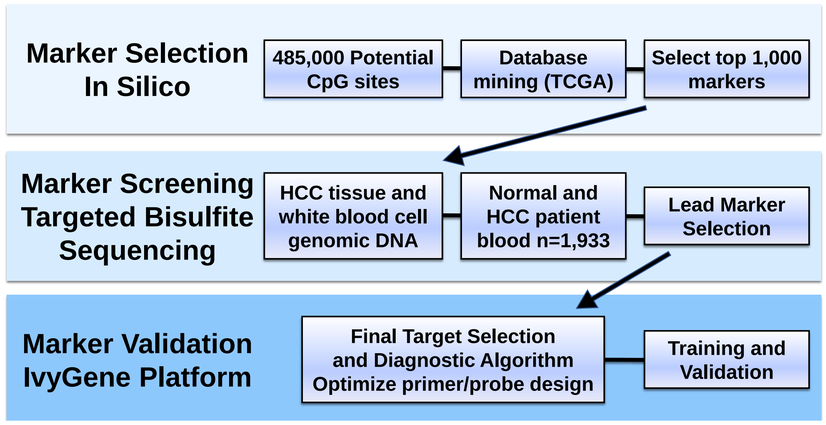
Fig 3. Oncogenesis Alters DNA Methylation Patterns



- DNA methylation is an epigenetic regulator of gene expression
- Cytosine (C) may be methylated to form 5-methylcytosine (5mC) when 5' adjacent to Guanosine (G). These dinucleotide sites are called CpG sites
- Clusters of CpG sites (CpG islands) are found in gene regulatory regions
- Select alterations in DNA methylation consistently occur for some and perhaps all cancers

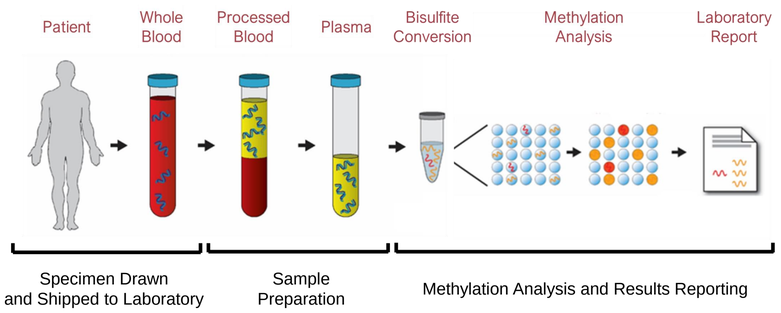
Methods

Fig 4. Marker Selection, Screening and Validation



- Initial marker screening performed using cfDNA from 1,933 patient samples[3]
- Final targets were selected and adapted for use on the IvyGene Platform

Fig 5. The IvyGene Platform



- The IvyGene Platform consists of methods and procedures for the collection, processing and quantitative analysis of methylation of cell-free DNA
- The IvyGene Dx Liver Cancer Test measures DNA methylation at select CpG sites that highly correlate with hepatocellular carcinoma (HCC)

Table 1. Validation Study Cohort

	Normal	Benign Liver Disease	Liver Cancer (HCC)	Breast Cancer	Colon Cancer	Lung Cancer
Total	30	10	60	10	10	10
Stage I	-	-	9	1	1	1
Stage II	-	-	10	5	2	5
Stage III	-	-	34	2	2	1
Stage IV	-	-	7	2	5	3

- Normal, healthy subjects had no symptoms or history of cancer
- Benign liver diseases included patients diagnosed with: cirrhosis (1), HBV (2) benign liver nodule (3) and hepatic cyst (4)
- All cancer patients were diagnosed according to current medical practice
- All liver cancer patients were diagnosed with hepatocellular carcinoma (HCC)

Results

Fig 6. Validation Study of the IvyGene Dx Liver Cancer Test

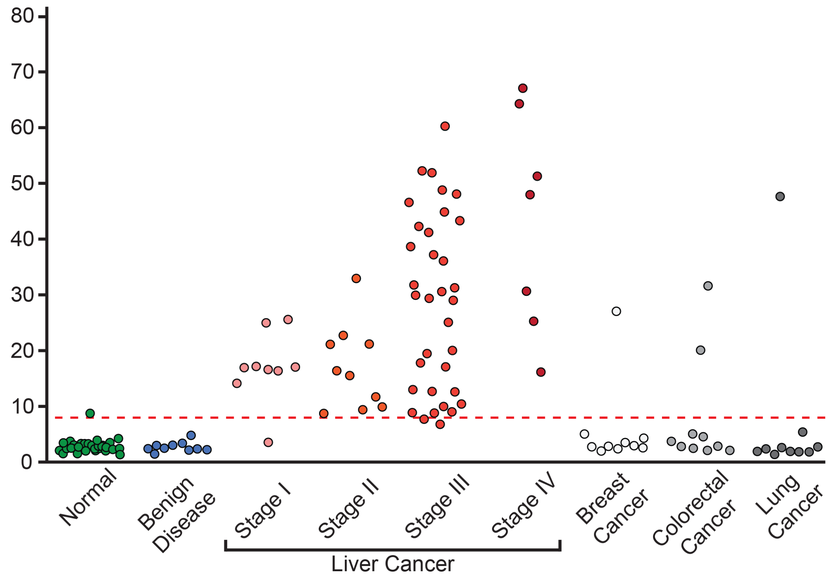


Table 2. Test Performance

			Hepatocellular Carcinoma				Breast Colorectal & Lung
	Normal	Benign	Stage I	Stage II	Stage III	Stage IV	
Total	30	10	9	10	34	7	30
Positive	1	0	8	10	32	7	4
Negative	29	10	1	0	2	0	26
Sensitivity	-	-	89%	100%	94%	100%	-
Specificity	97%	100%	-	-	-	-	87%

Table 3. Comparison to Current HCC Imaging Techniques

	Sensitivity (95% CI)	Specificity (95% CI)
IvyGene Dx Liver Cancer test	95% (89-100%)	98% (93-100%)
Ultrasound[4]	73% (46-90%)	93% (85-97%)
MRI[4]	86% (79-91%)	89% (82-93%)
CT[4]	83% (76-88%)	91% (84-95%)

Conclusions

- The IvyGene Dx Liver Cancer test has been developed to detect HCC at an early stage with a high degree of sensitivity and specificity
- Benign disease can be differentiated from HCC by quantifying methylation of cfDNA
- Patient outcomes are predicted to improve by detecting liver cancers at an early, localized stage

References:

1. World Health Organization. GLOBOCAN 2018. Graph production: IARC (http://gco.iarc.fr/today)
2. Fast Stats: An interactive tool for access to SEER cancer statistics. Surveillance Research Program, National Cancer Institute. https://seer.cancer.gov/faststats. (Accessed on 11-2-2018)
3. Xu, R. H., et. al. (2017) Circulating tumor DNA methylation markers for diagnosis and prognosis of hepatocellular carcinoma. Nature materials 16, 1155-1161.
4. Chou, R., et. al. (2015) Imaging Techniques for the Diagnosis of Hepatocellular Carcinoma: A Systematic Review and Meta-analysis. Annals of internal medicine 162, 697-711

Human Subjects:

This project was approved by the Institutional Review Boards (IRBs) of Sun Yat-sen University Cancer Center, Xijing Hospital, and West China Hospital. Informed consent was obtained from all patients.