Epigenetic biomarker discovery and validation for diagnosis and therapeutic intervention for Hepatocellular Carcinoma

Xueguang Sun, Jill E. Petrisko, Lam K. Nguyen, Marc Van Eden & Xi-Yu Jia

Zymo Research Corporation, Irvine, CA

ZYMO RESEARCH

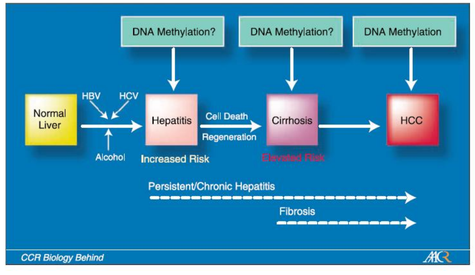
The Beauty of Science is to Make Things Simple

Abstract

Hepatocellular carcinoma (HCC) is one of the most common and lethal malignancies worldwide, accounting for approximately half a million annual deaths globally [1]. HCC is completely asymptomatic in the early stages of the disease; therefore, early detection of HCC in afflicted patients is vital to receive therapeutic benefits from curative surgery. The standard diagnosis of HCC relies upon detection of the serum alpha-fetoprotein (AFP) level in at-risk subjects followed by hepatic ultrasonography to identify suspicious nodules [2]. Accurate levels of AFP are often difficult to detect, and the imaging method to identify nodules is both operator-sensitive and subject to a high false-negative rate. While it is known that hepatocellular carcinoma is a multi-step process that requires altered expression of multiple genes, recent evidence has indicated that epigenetic abnormalities also play an important role in HCC. The discovery of reliable and accurate epigenetic biomarkers may open up new avenues for the development of novel diagnostic tools and provide a new target for therapeutic interventions.

Background

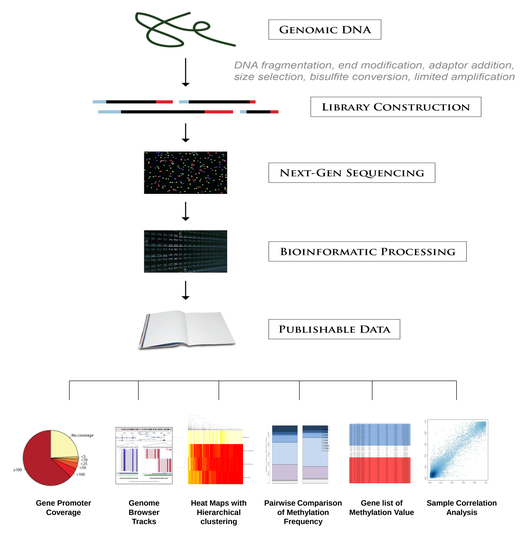
The etiology of HCC is multifactorial, and several agents have been implicated in the development of chronic liver disease leading to cancer, including alcohol consumption, and chronic infection with hepatitis viruses [3]. Persistent/chronic hepatitis is a pathological condition characterized by hepatocyte cell death. With disease progression, patients with chronic hepatitis will develop cirrhosis. Cirrhosis, as a preneoplastic conditon of the liver, is the major risk factor for HCC in humans. Molecular pathogenesis of HCC involves genetic and epigenetic events. Aberrant DNA methylation is known to occur in HCC, but may also be important in preneoplastic lesions that lead to cancer development. Therefore, epigenetic biomarkers may be useful for early detection of both preneoplastic lesions and early cancer development among high-risk populations.



Methodology

Combining Next Generation Sequencing methods with well-established bisulfite conversation chemistry, a unique genome-wide epigenetic profile for HCC was done in liver tumor and normal tissues. Hypermethylated and hypomethylated gene regions from the genome-wide epigenetic profile were then validated for percent methylation in liver tumor and normal tissues using a streamlined system of methylation-sensitive restriction enzyme (MSRE) digestion combined with real-time PCR. Serum circulated genomic DNA from liver cancer patients _was also examined to look for potential epigenetic markers for clinical diagnostic application.

Workflow



- Next-Gen sequencing based platforms
- Low DNA input (as low as 50 ng)
- Applicable to broad range of sample sources and species
- Streamlined workflows with comprehensive bioinformatic analysis.

Results and Discussion

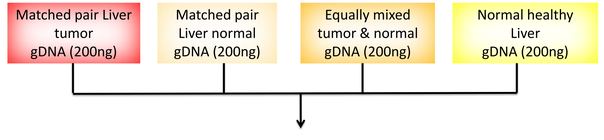


Figure 1. Genomic DNA was isolated from matched pair liver tumor and normal adjacent tissue samples, a mixture of matched pair liver tumor and normal adjacent samples, and normal healthy liver samples. All samples were investigated using the workflow shown above.

➢ **Genome Coverage :**

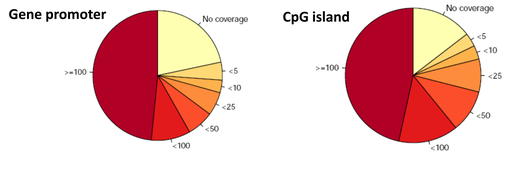


Figure 2. Graphs show the overall distribution of sequencing read at gene promoters and CpG islands

Results

➢ **Methylation Index**

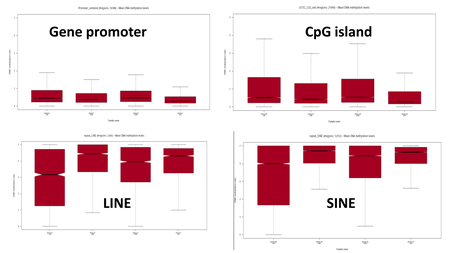


Figure 3. The amount of global DNA methylation (methylation index) in matched pair liver tumor samples, matched pair normal adjacent liver samples, a mixture of matched pair tumor and normal liver samples, and healthy liver samples. DNA repeats (LINEs and SINEs) undergo global hypomethylation in matched liver tumor samples compared with matched pair normal adjacent liver and healthy liver samples.

➢ **Number of Different Methylation Regions**

		Gene Promoter	CpG island	miRNA	LTR	LINE	SINE
Paired normal	Strong hypermethylation	277	653	6	5	3	16
	hypermethylation	2195	3059	14	4	0	1
	insignificant	12633	14997	142	2858	1896	8801
	hypomethylation	723	1317	9	66	9	29
	strong hypomethylation	215	704	4	738	128	1013
Normal	Strong hypermethylation	520	1173	14	36	17	53
	hypermethylation	4734	5642	32	15	1	7
	insignificant	10080	12536	123	2544	1621	7005
	hypomethylation	273	670	2	92	5	13
	strong hypomethylation	215	567	2	826	181	779
Tumor+Normal	Strong hypermethylation	144	302	5	1	0	3
	hypermethylation	1222	1908	7	1	0	0
	insignificant	13606	16714	154	3497	2015	9664
	hypomethylation	953	1443	8	36	1	8
	strong hypomethylation	129	373	1	162	24	175

Figure 4. More than one hundred gene loci were identified at differentially methylated regions (DMRs) by comparing several different stage liver tumors with their correspondent normal tissue by the genome wide epigenetic profile.

➢ **UCSC genome browser track**

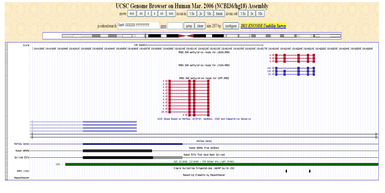


Figure 5. UCSC genome browser shows one locus with methylation value

➢ **Hierarchical clustering:**

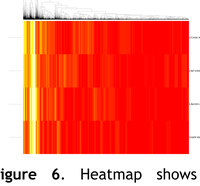


Figure 6. Heatmap shows the hierarchical clustering by region and sample.

➢ **Correlation analysis**

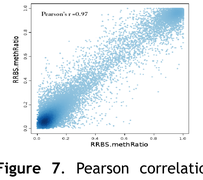


Figure 7. Pearson correlation for pairwise data comparison between samples

Results

➢ **Validation**

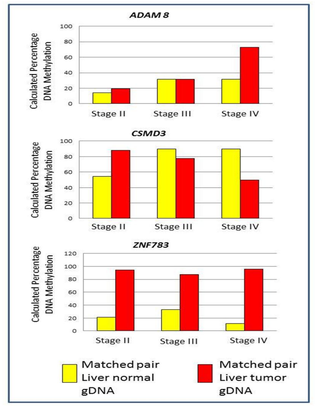


Figure 7. Calculated Ct values from combined MSRE digestion and real-time PCR quantitation indicated that percent methylation of the CpG island in the gene *ADAM8* was highest in stage IV liver tumor tissue, while percent methylation of the CpG island in *CSMD3* was lowest in stage IV tissue compared to normal adjacent tissue. Percent methylation of the CpG island in *ZNF783* was high in all stages of HCC tumor liver tissue compared to normal adjacent tissue.

Conclusions

- A Next-Gen sequencing based platform was used to screen for epigenetic biomarkers at the genomic scale for diagnosis and therapeutic intervention for heptatocellular cancer. More than one hundred gen loci were identified at differententially methylated regions.
- An increase in global hypomethylation in DNA repeats (LINES and SINEs) was observed in the matched liver tumor samples when compared with matched pair normal adjacent liver and healthy liver samples.
- The gene *ADAM8* was highest in stage IV liver tumor tissue, while percent methylation of the CpG island in CSMD3 was lowest in stage IV tissue compared to normal adjacent tissue. Percent methylation of the CpG island in ZNF783 was high in all stages of HCC tumor liver tissue compared to normal adjacent tissue

References

[1] El-Serag HB, Rudolph KL (2007) Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 132:2557–2576

[2] Patel M, Shariff MI, Ladep NG, Thillainayagam AV, Thomas HC, Khan SA, Taylor-Robinson SD. Hepatocelluar carcinoma: diagnostics and screening. J Eval Clin Pract. 2012 Apr;18(2):335-42.

[3] Rivenbark AG, Coleman WB. The use of epigenetic biomarkers for preclinical detection of hepatocellular carcinoma: potential for noninvasive screening of high-risk populations. Clin Cancer Res. 2007 Apr 15;13(8):2309-12

[4] Gu H, Smith ZD, Bock C, Boyle P, Gnirke A, **Meissner A**. Preparation of reduced representation bisulfite sequencing libraries for genome-scale DNA methylation profiling. Nat Protoc. 2011 Apr;6(4):468-81