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Identification of a Panel of Epigenetic Biomarkers for Early Detection of Hepatocellular Carcinoma in Blood of Individuals with Liver Cirrhosis

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Abstract : Hepatocellular carcinoma (HCC), the most prevalent type of primary liver cancer, is the second leading cause of cancer death worldwide. Late onset of clinical symptoms in HCC results in late diagnosis and poor disease outcome. Approximately 85% of individuals with HCC have underlying liver cirrhosis. However, not all cirrhotic patients develop cancer. Reliable early detection biomarkers that can distinguish cirrhotic patients who will develop cancer from those who will not are urgently needed and could increase the cure rate from 5% to 80%. We used Illumina-450K microarray to test whether blood DNA, an easily accessible source of DNA, bear site-specific changes in DNA methylation in response to HCC before diagnosis with conventional tools (pre-diagnostic). Top 11 differentially methylated sites were selected for validation by pyrosequencing. The diagnostic potential of the 11 pyrosequenced probes was tested in blood samples from a prospective cohort of cirrhotic patients. We identified 971 differentially methylated CpG sites in pre-diagnostic HCC cases as compared with healthy controls ($P < 0.05$, paired Wilcoxon test, ICC ≥ 0.5). Nearly 76% of differentially methylated CpG sites showed lower levels of methylation in cases vs. controls ($P = 2.973E-11$, Wilcoxon test). Classification of the CpG sites according to their location relative to CpG islands and transcription start site revealed that those hypomethylated loci are located in regulatory regions important for gene transcription such as CpG island shores, promoters, and 5'UTR at higher frequency than hypermethylated sites. Among 735 CpG sites hypomethylated in cases vs. controls, 482 sites were assigned to gene coding regions whereas 236 hypermethylated sites corresponded to 160 genes. Bioinformatics analysis using GO, KEGG and DAVID knowledgebase indicate that differentially methylated CpG sites are located in genes associated with functions that are essential for gene transcription, cell adhesion, cell migration, and regulation of signal transduction pathways. Taking into account the magnitude of the difference, statistical significance, location, and consistency across the majority of matched pairs case-control, we selected 11 CpG loci corresponding to 10 genes for further validation by pyrosequencing. We established that methylation of CpG sites within 5 out of those 10 genes distinguish cirrhotic patients who subsequently developed HCC from those who stayed cancer free (cirrhotic controls), demonstrating potential as biomarkers of early detection in populations at risk. The best predictive value was detected for CpGs located within BARD1 (AUC=0.70, asymptotic significance ˂0.01). Using an additive logistic regression model, we further showed that 9 CpG loci within those 5 genes, that were covered in pyrosequenced probes, constitute a panel with high diagnostic accuracy (AUC=0.887; 95% CI:0.80-0.98). The panel was able to distinguish pre-diagnostic cases from cirrhotic controls free of cancer with 88% sensitivity at 70% specificity. Using blood as a minimally invasive material and pyrosequencing as a straightforward quantitative method, the established biomarker panel has high potential to be developed into a routine clinical test after validation in larger cohorts. This study was supported by Showalter Trust, American Cancer Society (IRG#14-190-56), and Purdue Center for Cancer Research (P30 CA023168) granted to BS.

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