

Hypermethylation of SCAND3 and Myo1g Gene Are Potential Diagnostic Biomarkers for Hepatocellular Carcinoma

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Table S1. Clinicopathological characteristics of the 20 HCC patients.

Characteristics	Values
Sex	
Male	16
Female	4
Age (years)	53.16 ± 4.17
Tumor number	
Single	15
Multiple	5
BCLC stage	
0	8
A	7
B	5
C	0
HBV	
Positive	14
Negative	6
Liver cirrhosis	
Positive	7
Negative	13

Methylation Data Analysis

For digital droplet PCR (ddPCR), after PCR amplification, the 96-well PCR plates were placed into the Bio-Rad QX200 droplet reader and data were analyzed using QuantaSoft software (Bio-rad). The absolute quantification (ABS) experiment design was used in the data analysis. The number of positive and negative droplets in each sample was counted by QuantaSoft software. In our present study, the positive droplets (P) were regarded as a targeted gene (SCAND3 or Myo1g) methylated, the negative droplets (N) were regarded as reference gene GAPDH. The ratio between the number of P and the total number of P + N was used to represent the absolute methylation level for each sample. In other word, the percentage of methylation for each sample was calculated as % methylation = $100 \times [P / (N + P)]$.

For methylation-specific PCR (MSP), the optimal cutoff value of CT (cycle threshold) in MSP assay for SCAND3 and Myo1g methylation was established based on the receiver operating characteristics curve (ROC) analysis [1-3]. All tested samples were categorized as methylated or unmethylated based on the cutoff values. The optimal cutoff of CT value for SCAND3 was 20. If CT value was < 20 cycles, a sample was considered as SCAND3 methylated; if CT value was ≥ 20 or not measurable, a sample was considered as SCAND3 unmethylated. The optimal cutoff of CT value for Myo1g was 23. If CT value was < 23 cycles, a sample was considered as Myo1g methylated; if CT value was ≥ 23 or not measurable, a sample was considered as Myo1g unmethylated. The test result of each sample was acceptable only when the CT value of the reference gene GAPDH was ≤ 30 .

References

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