Supplemental materials

Supplementary experimental procedure

Development of custom algorithm for MeDIP-chip analysis

From the scanned microarray data obtained from hybridization of the immunoprecipitated (IP) DNA and the total DNA (whole cell extract DNA, WCE) labeled with Cy5 and Cy3, respectively, signal values of the IP and WCE were normalized using background subtraction, then the signal log ratio \[
[\log_2(\text{IP/WCE})]
\] was calculated.

Among CpG islands on the CpG island microarray (Agilent), we focused on those spanning both an upstream region and the body of each gene. To determine the cut value for evaluation of methylation status, we selected 22 genes whose methylation status in at least one of three hepatoma cell lines (Hep3B, HepG2, and HuH-7) was analyzed using COBRA, BGS, or methylation-specific PCR (MSP) in previous studies (S1-S18) or obtained in our preliminary experiments (Supplementary Table S3). Using this gene set, signal log ratio of probes within CpG islands were compared with the known methylation status. The pattern of output values of probes within CpG islands of each gene in the gene set was correlated with methylation status, and the highest value among output values of all probes within each CpG island showed a good correlation with methylation status. Based on a receiver operating characteristic (ROC) curve obtained by plotting the true positive rate and false positive rate in this gene set, we determined 1.0 as a cutpoint of the highest value of signal log ratio within each CpG island with high sensitivity (88.5%) and high specificity (62.5%). Therefore, genes having probes with the highest value of signal log ratio \(\geq 1.0\) were determined as methylated, whereas genes having probes with the highest value of signal log ratio \(< 1.0\) were determined as unmethylated.

Supplementary references


MZB1 methylation in hepatocellular carcinoma


Supplementary figure legends

Supplementary figure S1

Determination of the cut-off value to accurately distinguish methylated genes in MeDIP-chip analyses.

A, distribution and frequency of the highest signal log ratio \([\log_2(\text{IP}/\text{WCE})]\) within CpG islands of 22 genes in three hepatoma cell lines obtained by MeDIP-chip analyses (see Supplementary Table S4). The methylation status of genes in hepatoma cell lines was determined using data from references (S1-S18). Notably, genes expected to be methylated (right) had higher values than genes expected to be unmethylated (left).

B, a receiver-operator characteristic (ROC) curve (upper) and sensitivity, specificity, false positive rate in each cut-off value (lower) of the signal log ratio \([\log_2(\text{IP}/\text{WCE})]\) for discriminating methylated genes using known methylation status of 22 genes in three hepatoma cell lines. At a cut-off value of 1.0, sensitivity and specificity were 88.5 and 62.5, respectively.

Supplementary figure S2

Profiles of relative expression of 11 candidates determined by quantitative real-time RT-PCR in three hepatoma cell lines (Hep3B, HepG2, and HuH-7) treated with 1, 5, and 10 μM of 5-aza-dCyd. Ratio relative to non-treated cells was shown in a logarithmic scale.

Supplementary figure S3

Representative results of the methylation status of Region 3 of FAR1 CpG-island determined by COBRA (upper) and the relative expression level of FAR1 mRNA determined by quantitative real-time RT-PCR (lower) in surgically resected primary HCC tumors (T) and corresponding non-cancerous liver tissues (N). Closed circles indicate samples with the restricted fragments from methylated alleles (methylation positive) in a tumor-specific manner, whereas open circles indicate those with reduced mRNA expression (>50% decreased expression) in tumor tissue compared with paired non-tumorous tissue.
Supplementary figure S4

Representative results of BGS of the MZB1 CpG island (Region 1-8) in HLE cells without and with 10 μM 5-aza-dCyd. See legend for Figure 2C.