## Abstract

### Introduction

Gastric cancer is divided into four subtypes by their molecular features linked with genetic alterations, e.g., Epstein-Barr virus (EBV), microsatellite instability-high (MSI-high), chromosomal instability (CIN), and genomically stable (GS), called as TCGA classification. In this study, we tried to clarify the epigenetic features of the four GC subtypes according to aberrant methylation status in 23 loci.

### Methods

A total of 98 gastric cancers and their normal gastric mucosa samples were included in this study. We divided gastric cancers into TCGA subtypes which were determined in line with MSI-high, EBV, CIN, to GS by their molecular features. The 13 loci of polymorphic microsatellite sequences were used to determine loss of heterogeneity (LOH) for the detection of CIN. The MSI status was determined by three mononucleotide repeat markers. Infection of EBV was determined by recovering EBV *BNRF1* sequence from genomic DNA collected from gastric cancers. Methylation status of 23 loci was investigated by the combined bisulfite restriction analysis (COBRA). Status of other findings, e.g., *KRAS* mutations, HER2 expression status and infection of helicobacter pylori were confirmed.

### Results

Gastric cancers were divided into MSI (13%), EBV (7%), CIN (53%), and GS (27%). By histological classification, poorly differentiated adenocarcinoma (por) was more in tumors categorized in MSI-high, and GS and signet-ring cell carcinoma (sig) was more in GS. Among the 23 loci investigated their methylation status, 18 loci were significantly hypermethylated in caner tissues. A unsupervised clustering divided gastric cancers into two clusters, and revealed that most GS tumors clustered together in a cluster that exhibited lower methylation levels, distinct from the other subtypes. The inter-variable clustering revealed that a cluster contained the three loci (*SFRP2*-region 1/2 and APC) belonging to the Wnt signal cascade (Wnt-associated loci). The mean methylation score of Wnt-associated loci was the lowest in GS tumors (MSI-high: 2.7 [95% confidence interval (CI), 2.3-2.9]; EBV:2.1[1.2-3.1]; CIN: 2.4 [2.2-2.7]; GS: 1.3 [0.8-0.7]). In contrast, the mean methylation score of the other 15 loci was significantly higher in MSI-high, while that in GS was as same as that in EBV or CIN (MSI- high: 10.4 [8.3-12.4]; EBV:5.7 [1.7-9.7]; CIN: 4.4 [3.6-5.1]; GS: 3.4 [2.2-4.6]). Additionally, the lower methylation score of Wnt-associated loci was observed only in sig tumors.

### Conclusions

GS subtype tumors have the potential to possess distinct signatures in DNA hypomethylation profiles in Wnt signaling pathway, especially in signet-ring cell carcinoma.