Using methylation-specific real-time PCR, we determined the prevalence of aberrant methylation in the mismatch repair gene hMLH1 and in the recently described HPP1 gene among 50 esophageal, 50 cardiac and 50 gastric ADCs. Additionally, expression of hMLH1 protein was detected immunohistochemically and correlated with DNA MSI. Hypermethylation of hMLH1 was found in 14% of esophageal, 28% of cardiac and 32% of gastric ADCs, whereas HPP1 hypermethylation was found more frequently in the 3 tumor types (64% vs. 38% vs. 54%). In gastric ADC, HPP1 hypermethylation was found more frequently in tumors with concomitant hMLH1 hypermethylation (81%) than in those without hMLH1 hypermethylation (41%, p = 0.008). Complete loss of hMLH1 protein expression, which was present in 10 carcinomas (5 cardiac and 5 gastric), was invariably correlated with hMLH1 hypermethylation and MSI. In conclusion, our data indicate that MSI and loss of the mismatch repair protein hMLH1, which is mainly caused by hMLH1 gene hypermethylation, are more prevalent in stomach and cardia carcinogenesis than in that of the esophagus. Moreover, in gastric cancer, hMLH1 hypermethylation is correlated with hypermethylation of the HPP1 gene.