Abstract:

PURPOSE: Promoter hypermethylation occurs frequently in tumors and leads to silencing of tumor-relevant genes like tumor suppressor genes. In a subset of sporadic colorectal cancers (CRC), inactivation of the mismatch repair gene MLH1 due to promoter methylation causes high level of microsatellite instability (MSI-H). MSI-H is also a hallmark of hereditary nonpolyposis colorectal cancer (HNPCC) in which mismatch repair inactivation results from germ-line mutations. For differentiation of sporadic and hereditary MSI-H tumor patients, MLH1 promoter methylation analysis is a promising tool but is not yet used in daily diagnostics because only qualitative techniques without standardization are available. The aim of this study is to establish a reliable and quantitative MLH1 methylation analysis technique and to define valid MLH1 methylation cutoff values for HNPCC diagnostics.

EXPERIMENTAL DESIGN: We developed a new real-time PCR-based technique to detect and quantify methylation of both proximal and distal hMLH1 promoter regions. We established and validated this technique in a cohort of 108 CRCs [94 MSI-H and 16 microsatellite stable (MSS) cases] comprising a reference (n = 58) and a tester tumor group (n = 50).

RESULTS: The reference tumor group
contained 28 HNPCC with proven germ-line mutations or positive Amsterdam I criteria (median age, 37 years) and loss of MLH1 expression, 14 sporadic MSI-H CRC tumors with loss of MLH1 expression and BRAF V600E mutation (median age, 80.5 years), and 16 sporadic MSS CRC (median age, 76.5 years). No MLH1 promoter methylation could be found in any MSS tumors. HNPCC patients showed no or low level of MLH1 promoter methylation. A cutoff value of 18% methylation extent could be determined in this study to define MLH1 hypermethylation specific for sporadic MSI-H cases. Methylation could also be verified qualitatively by melting point analysis. BRAF V600E mutations were not detected in any HNPCC patients (n = 22 informative cases). CONCLUSION: According to the present data, quantitative MLH1 methylation analysis in MSI-H CRC is a valuable molecular tool to distinguish between HNPCC and sporadic MSI-H CRC. The detection of a BRAF V600E mutation further supports the exclusion of HNPCC.