Colon cancer prognosis and treatment are currently based on a classification system still showing large heterogeneity in clinical outcome, especially in TNM stages II and III. Prognostic biomarkers for metastasis risk are warranted as development of distant recurrent disease mainly accounts for the high lethality rates of colon cancer. miRNAs have been proposed as potential biomarkers for cancer. Furthermore, a verified standard for normalization of the amount of input material in PCR-based relative quantification of miRNA expression is lacking. A selection of frozen tumor specimens from two independent patient cohorts with TNM stage II-III microsatellite stable primary adenocarcinomas was used for laser capture microdissection. Next-generation sequencing was performed on small RNAs isolated from colorectal tumors from the Dutch cohort (N = 50). Differential expression analysis, comparing in metastasized and nonmetastasized tumors, identified prognostic miRNAs. Validation was performed on colon tumors from the German cohort (N = 43) using quantitative PCR (qPCR). miR25-3p and miR339-5p were identified and validated as independent prognostic markers and used to construct a multivariate nomogram for metastasis risk prediction. The nomogram showed...
good probability prediction in validation. In addition, we recommend combination of miR16-5p and miR26a-5p as standard for normalization in qPCR of colon cancer tissue-derived miRNA expression. In this international study, we identified and validated a miRNA classifier in primary cancers, and propose a nomogram capable of predicting metastasis risk in microsatellite stable TNM stage II-III colon cancer. In conjunction with TNM staging, by means of a nomogram, this miRNA classifier may allow for personalized treatment decisions based on individual tumor characteristics.