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Correlation of polypoid colorectal adenocarcinoma with pre-existing adenomatous polyps and *KRAS* mutation

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Cetuximab is an anti–epidermal growth factor receptor that helps effectively treat patients with advanced colorectal adenocarcinoma without *KRAS* activating mutations. *KRAS* mutations are associated with 16% to 50% of isolated villous adenomas and approximately 30% of colorectal cancer. Correlation between the gross and histological subset of colorectal adenocarcinoma with *KRAS* mutation is unknown. Archived surgical resection specimens of colorectal adenocarcinoma ($n = 42$) and villous adenoma ($n = 9$) were collected. The gross appearance and histopathological features of these lesions were thoroughly reviewed, including the presence of a pre-existing adenomatous polyp. DNA was extracted from formalin-fixed, paraffin-embedded tissue sections and then subjected to TaqMan real-time polymerase chain reaction to detect the seven most common *KRAS* mutations. *KRAS* mutations were found in 13 of 42 cases (31%) of colorectal adenocarcinoma and 7 of 9 cases (78%) of villous adenoma. All 13 cases of colorectal carcinoma with a *KRAS* mutation showed a gross polypoid configuration, compared to no *KRAS* mutation in the colorectal carcinomas with ulcerative configuration. In addition, 13 of 17 of these cases (76%) had histological features of adenocarcinoma with a persistent preexisting adenomatous polyp with villous architecture. In summary, grossly polypoid colorectal adenocarcinomas with a persistent pre-existing adenomatous polyp with villous architecture are strongly associated with *KRAS* mutations.

Keywords *KRAS*, colorectal adenocarcinoma, villous adenoma, adenomatous polyp with villous architecture

Colorectal carcinoma is the second and third leading cause of cancer death for women and men, respectively, in the United States, despite recent progress in reducing its incidence and mortality rate (1). Most colorectal carcinomas can be described as having either a polypoid or ulcerative appearance on gross examination. The former presents as a well-defined bulky mass, whereas the latter has a less elevated surface and is often centrally ulcerated. In patients with colorectal carcinoma unresponsive to chemotherapy, monoclonal antibody therapies targeting the epidermal growth factor receptor (EGFR), such as cetuximab, have demonstrated improvement in the overall and progression-free survival, as well as preservation of the quality of life in this group of patients (2,3). However, recent studies report that patients with a colorectal tumor bearing a mutated *KRAS* gene do not benefit from cetuximab (4–8), with the exception of the *KRAS* Gly13Asp mutation (9).

The *KRAS* gene is one of the frequently mutated proto-oncogenes in colorectal tumors (10). KRAS is a signaling molecule downstream of EGFR, a transmembrane receptor for extracellular signaling, and upstream of RAF in the RAS/RAF/MAPK signaling pathway (11–13). Wild-type KRAS regulates signal transduction through its GTPase activity by switching the GDP-bound inactive form to the GTP-bound active form. Point mutations at codons 12 or 13 of *KRAS* abolish its GTPase activity and result in a constitutively active form of KRAS, irrespective of upstream signaling (14,15). Thus, in patients with colorectal carcinoma carrying a *KRAS* mutation at codons 12 or 13, blockage of upstream EGFR

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activation with EGFR inhibitors such as cetuximab will not affect the constitutively activated *KRAS* mutant and its effects on the downstream signaling pathways, which leads to the uncontrolled proliferation of tumor cells.

The association of the *KRAS* mutation at codons 12 or 13 with benign colorectal adenomatous polyps (including villous adenoma, tubulovillous adenoma, and tubular adenoma) has been reported in several studies (10,13–20). These mutations are more common in larger adenomas, in adenomas with villous morphology, and in adenomas with high-grade dysplasia. In these advanced adenomas, the *KRAS* mutation detection rate ranges from 28% to 50%. The association of the *KRAS* mutation with sex was not completely clear, but some studies revealed that *KRAS* mutations are unrelated to sex. Despite extensive studies on *KRAS* mutations in colorectal adenomas, the association of *KRAS* mutations with the gross and histologic subtypes of malignant colorectal adenocarcinoma remains unclear.

In this study, we investigated the characteristics of a subset of colorectal carcinomas carrying the *KRAS* mutation, including gross configuration, histological features, and patient characteristics. We then correlated these characteristics with the mutation status of the *KRAS* gene in an attempt to provide a better understanding of the importance of accurate conventional tissue examination in pre-treatment screening of colorectal carcinomas.

Materials and methods

Tissue samples and histologic examination

Archived surgical resection specimens of villous adenoma ($n = 9$) and colorectal adenocarcinoma samples from patients who did not receive neoadjuvant therapy ($n = 42$) were collected from the pathology files at the Dartmouth-Hitchcock Medical Center. This study was approved by the Committee for the Protection of Human Subjects at the Darmouth College and the Dartmouth Hitchcock Medical Center. Among the 42 cases of colorectal adenocarcinoma, 28 cases were randomly selected from June 2007 to June 2008; an additional 14 cases of colerectal adenocarcinoma with pre-existing adenomatous polyp with villous architecture were selectively chosen from 2004 to 2010. Nine cases of villous adenoma were randomly selected from 2005 to 2008. Normal colon sections ($n = 2$) from two cases of colorectal adenocarcinoma were used as negative controls. The histopathological features of these colorectal adenocarcinomas were thoroughly reviewed, including gross configuration, tumor types, differentiation, and the presence of persistent pre-existing adenomatous polyps. The 42 cases of colorectal adenocarcinoma included 17 cases of colorectal adenocarcinoma arising in association with adenomatous polyp with villous architecture (including villous adenoma and tubulovillous adenoma) and 25 cases of colorectal adenocarcinoma without pre-existing adenomatous polyp with villous architecture.

DNA extraction

Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue sections (five 10-μm sections) with macrodissection in colorectal adenocarcinoma cases treated in the years 2009–2010 ($n = 8$) and without macrodissection for the rest of cases. Each tissue block was carefully selected so that the tissue analyzed contained at least 50% tumor for this study. Tissue sections were collected in microcentrifuge tubes, deparaffinized with 2–3 1.0 mL xylene washes, then washed in 100% ethanol twice, once in 95% ethanol and once in phosphate-buffered saline. Genomic DNA was then purified from these tissue specimens by using Gentra Puregene reagents and protocol (Qiagen, Valencia, CA).

KRAS mutation analysis

Each DNA sample was subjected to seven separate real-time polymerase chain reaction (PCR) amplifications using primer–probe sets designed to detect seven common *KRAS* mutations found in codons 12 and 13 (Gly12Arg, Gly12Asp, Gly12Cys, Gly12Ala, Gly12Ser, Gly12Val, and Gly13Asp). Each PCR contained a pair of oligonucleotide primers flanking codons 12 and 13 along with one wild-type oligonucleotide probe fluorescently labeled with VIC and another probe specific for one of the seven mutations labeled with FAM (21). Each TaqMan assay included testing synthetic oligonucleotide controls for the specific *KRAS* mutation and wild-type sequences. All primers and probes were synthesized by Applied Biosystems and were used at final concentrations of 900 nmol/L and 200 nmol/L, respectively, in 1× TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA) with 5–20 ng of genomic DNA on a 7500 Fast Real-time PCR System. Allelic discrimination analysis was performed by 7500 software, version 2.0 (Applied Biosystems). Representative DNA samples, positive and negative for *KRAS* mutations, were also subjected to Sanger DNA sequencing of the first coding exon of *KRAS* with the CEQ 8000 Genetic Analysis System (Beckman Coulter, Brea, CA) to confirm real-time PCR results.

Statistical analysis

The numbers of cases that tested positive for a *KRAS* mutation between any two selected groups in the study were compared by the non-paired Student *t*-test. A *P*-value of less than 0.01 was considered statistically significant. The statistical analysis delineated the differences in percentage of *KRAS* mutations between groups of colorectal carcinoma with different gross and histologic features.

Results

In this study, the patients' age ranged from 33 to 95 years with the mean age of 68 years. The patient population and tumor characteristics are summarized in Table 1. There were no significant differences in patient age, tumor size, and tumor stage between patients with colorectal adenocarcinoma arising in association with adenomatous polyp with villous architecture and colorectal adenocarcinoma without pre-existing adenomatous polyp with villous architecture.

Of the 51 tumor tissues analyzed by TaqMan real-time PCR assays, 20 were identified as having *KRAS* mutations. There were 16 mutations in codon 12, including 8 GGT to