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Original contribution

Adenoma and carcinoma components in colonic tumors show discordance for *KRAS* mutation ☆

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Summary Activating mutations in *KRAS* are common events in the pathogenesis of colorectal carcinoma and predict response to treatment with anti-EGFR antibodies. Molecular pathology testing for *KRAS* mutations has become the standard of practice for patients with metastatic colorectal carcinoma. Despite the known histologic and molecular differences between adenomas and carcinomas, the concordance of *KRAS* mutation between adenomas and carcinomas has not been established leaving some open questions regarding the appropriate choice of tissue for *KRAS* mutation analysis and correct interpretation of the test results. To address these questions, we analyzed the concordance of *KRAS* mutation in 70 tumors that contained both adenoma and carcinoma components (2 cases of intramucosal carcinoma, 66 cases with invasion of the submucosa, and 2 invading the muscularis propria). For each case, DNA was separately isolated from the adenoma and the carcinoma component and analyzed for *KRAS* mutation using direct sequencing. Overall, 30 (43%) of the adenoma cases and 36 (51%) of the carcinoma cases were positive for *KRAS* mutation. Of the 70 cases, 16 (23%) showed discordant results. Interestingly, the fraction of discordant cases went down as the depth of carcinoma invasion increased. In summary, we identified significant *KRAS* mutation discordance between the adenoma and carcinoma component of the lesion. Our results suggest that effort should be made to analyze only the invasive component of the lesion and that caution should be taken when interpreting a result based on DNA extracted from noninvasive elements.

1. Introduction

Colorectal carcinoma is a common malignancy as well as a significant cause of morbidity and mortality worldwide. In 2008, colorectal carcinoma resulted in more than 608 000 deaths worldwide, making it the fourth most common cause of cancer-related death [1]. In the United States, colorectal carcinoma is the third most commonly diagnosed and the

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third leading cause of cancer-related death in both men and women. The current view held is that most sporadic cases of colorectal carcinoma originate from adenomatous polyps, through a multistep histologic process. Early detection and treatment of the neoplasm at the adenoma stage are considered to be responsible for the reduced mortality rate from colon carcinoma in the past few decades [2,3]. In a recent report of more than 2600 patients who were followed up for up to 23 years, resection of adenomas was associated with 53% reduction in mortality from colon carcinoma [2].

Differences between adenoma and carcinoma can be detected in both the molecular and the histomorphometric level. The process of adenoma-carcinoma sequence is accompanied by a continuous gain of somatic gene mutations. In the last 2 decades, there has been some major progress in our understanding of the molecular events underlying this carcinogenic process in the colon. The common genetic alterations found in colon carcinoma include inactivation of tumor suppressor genes such as *APC, TP53*, *SMAD4* and *DCC* as well as activating mutations in the *KRAS* and *NRAS* oncogenes [4,5]. Certain mutations such as activating mutations in *KRAS* and *NRAS* predict response to anti-EGFR treatment, and molecular testing for these mutations has become the standard of practice in patients with metastatic colorectal carcinoma [6]. Moreover, recent analysis of large clinical trials suggests a detrimental effect of anti-EGFR treatment for patients with *RAS* mutations [7].

The potential molecular differences between adenomas and carcinomas may have some important implications for clinical practice. For example, in adenomas with carcinoma component, it is not clear whether we can test the entire sample for *KRAS* mutations or should we first isolate the carcinoma cells and restrict the analysis to this population. In addition, it is yet to be established whether a superficial biopsy of a large colonic lesion accurately represents the mutation status in the invasive part of the tumor. The purpose of the present study is to examine the concordance of *KRAS* mutation between adenoma and carcinoma components of the same lesion.

2. Material and methods

2.1. Samples

Seventy archived cases of colorectal lesions containing adenomatous polyp with early carcinoma components were used in this study. Diagnoses were based on the World Health Organization diagnostic criteria [8]. The samples were achieved via international collaboration and were collected between the years 2010 and 2013 at the departments of pathology at Rambam Health Care Campus (Haifa, Israel) and the institute of pathology at Klinikum Bayreuth (Bayreuth, Germany). Each sample was reviewed by an expert gastrointestinal pathologist, and the adenoma and carcinoma areas in each case were marked. Only lesions containing adenoma and carcinoma in continuity were analyzed. The areas used for DNA extraction contained most of the adenoma and most of the carcinoma components in each sample, and each had 80% or more tumor cells. The study was approved by the local ethics committees.

2.2. DNA extraction

For each case, the adenoma and carcinoma areas marked by the gastrointestinal pathologist were microscopically dissected, and DNA was extracted using the QuickExtract FFPE DNA extraction kit (Epicentre, Madison, WI) according to manufacturer instruction. After treatment with Ribonuclease A (Qiagen, Hilden, Germany), DNA was purified using the GeneJET PCR Purification Kit (Thermo Scientific, Waltham, MA).

2.3. *KRAS* mutation analysis

For the analysis of concordance between the adenoma and carcinoma components, we analyzed *KRAS* codons 12 and 13, the most common site for mutation. DNA extracted from the tissue samples was used as a template for amplifying the region containing codons 12 and 13 of *KRAS*, as described previously [9]. Briefly, primers used for the PCR reaction were forward 5′-GGCCTGCTGAAAATGACTGAA-3′ and reverse 5′-GGTCCTGCACCAGTAATATGCA-3′. Amplicons were purified using the GeneJET PCR Purification Kit (Thermo Scientific, Waltham, MA) and subjected to bidirectional DNA sequencing using the BigDye terminator v1.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA) on an automated sequencer (ABI Prism 3130xl Genetic Analyzer; Applied Biosystems). Analysis of sequencing results was done using the Sequencher software (Gene Codes Corporation, Ann Arbor, MI). This mutation detection method was previously demonstrated sensitive for the identification of mutations present in 10% or more of the DNA copies [9]. We compared *KRAS* mutation between the adenoma and carcinoma component of each sample.

3. Results

Of the cases with available age and sex data, 38 (58%) were males and 28 (42%) were females, and the average age was 65 ± 11 years. The distribution of lesion locations was 13%, 8%, 52%, and 27% for the right colon, left colon, sigma, and rectum, respectively. Histologically, 2 lesions (3%) contained intramucosal carcinoma, 66 lesions (94%) were T1 stage, and 2 lesions (3%) were T2 stage. Of the 66 T1 stage lesions, 37 (56%) showed invasion to the upper third of the submucosal layer (SM1), 25 (38%) invaded the middle third of the submucosa (SM2), and 4 (6%) invaded