Pathology – Research and Practice 207 (2011) 399–402

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.de/prp

Teaching cases

Morphological and molecular heterogeneity in colorectal neoplasms with *K-RAS* mutation. A report of two cases

Hans Bösmüller[a,*], Wolfgang Kranewitter[b], Gerald Webersinke[b], Holger Rumpold[c], Margit Hackl[d], Falko Fend[e]

[a] *Institute of Pathology, Krankenhaus Barmherzige Schwestern Linz, Seilerstätte 4, A-4020 Linz, Austria*
[b] *Laboratory for Molecular Biology and Tumorcytogenetics, I. Internal Department, Krankenhaus Barmherzige Schwestern Linz, Austria*
[c] *I. Internal Department, Krankenhaus Barmherzige Schwestern Linz, Austria*
[d] *Institute of Pathology, Klinikum Mostviertel Amstetten, Austria*
[e] *Institute of Pathology, University of Tübingen, Germany*

ARTICLE INFO

Article history:
Received 6 September 2010
Received in revised form 26 February 2011
Accepted 29 March 2011

Keywords:
K-RAS
Heterogeneity
Colorectal cancer
Signet ring cell carcinoma

ABSTRACT

Approximately forty percent of colorectal cancers (CRC) are characterized by activating mutations of the *K-RAS* gene. Determination of *K-RAS* mutational status as a predictive marker for anti-EGFR therapy is usually based on the assumption of intratumoral homogeneity. We present two cases of CRC in which morphologically distinct tumor components were associated with different activating mutations of *K-RAS* in one patient and a mutated and a non-mutated portion in the second patient, as demonstrated by laser microdissection and consecutive molecular analyses.

Introduction

Approximately eighty-five percent of CRC are microsatellite-stable tumors and have their origins in adenomas or aberrant crypt foci, mostly initiated by an inactivating mutation of the *APC* tumor suppressor gene, resulting in constitutive activation of the WNT pathway [10,30]. The *K-RAS* mutation rate was evaluated between 27% and 64% [4,24], depending on stage and tumor type, but regardless of its evidence in colorectal adenomas, an average rate of 40% for CRC's was most often published [16]. Activating mutations of the *K-RAS* gene are considered to be an early stage event [27,32], and *K-RAS* mutations, in general, are associated with more advanced disease [6,8,24,28], higher metastatic potential, and poorer survival, although its prognostic value is currently debated intensively [9]. Mutations in codon 13 have biological relevance in terms of higher tumor stage and worse clinical outcome [6,11,16], and G12V mutations are regarded as an indicator of higher aggressiveness and shorter survival [2,3,16,24], a fact that Finkelstein only could not support [11]. Bazan was the only author who found a relation between codon 12 mutations and a mucinous histological tumor-type [7].

Since the introduction of EGF-receptor-targeted therapy, determination of *K-RAS* mutational status has gained practical relevance and is obligatory before initiation of therapy with the anti-EGFR antibody Cetuximab, since only CRCs carrying wild-type *K-RAS* show a clinical response in a proportion of cases [20,21].

Although tissue of invasive carcinoma with >30% tumor cells is considered sufficient for mutational analysis irrespective of the detection method, the potential impact of intratumoral heterogeneity for predictive testing has received little attention. Possibly due to different technical approaches, there is some controversy about the frequency of intratumoral heterogeneity of *K-RAS* mutations. Kimura et al. described intratumoral heterogeneity (IH) with different single mutations in 7% of colorectal cancer carrying *K-RAS* mutations, which did not occur on the same allele [19]. Intratumoral heterogeneity seems to be more frequent in early CRC and is encountered only rarely in advanced lesions, possibly due to clonal selection [22]. However, intratumoral heterogeneity in the sense of having different tumor areas with and without *K-RAS* mutations is supposed to be practically non-existent, suggesting a growth advantage for mutated clones; case two suggests that this co-existence may be a rare event.

A special type of heterogeneity was described by Tortola et al. [29]. She compared samples of mutated primary CRC with aspirates of bone marrow showing disseminated tumor cells with early subclones, while the primary clone was overgrown by a different and more aggressive subclone. The evidence of predominant mutations

* Corresponding author. Tel.: +43 732 7677 4742; fax: +43 732 7677 7318.
E-mail address: hans-christian.boesmueller@bhs.at (H. Bösmüller).

0344-0338/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.prp.2011.03.004

of codon 13 in the bone marrow indicated an early event of carcinogenesis, because this mutation type is frequently found in aberrant crypt foci. The clinical value of dissemination to the bone marrow remains unclear.

In our hospital, so far, 925 patients have been evaluated for the *K-RAS* status, of whom 231 had multiple sampling concerning carcinoma, neighboring adenoma, lymph node metastasis, and distant metastasis. We found two cases only that were not homogenous, representing 0.21% of the whole collective and 0.86% of the patients with multiple sampling. Nevertheless, investigation of intratumoral heterogeneity of the *K-RAS* status can be of practical value for tumors with morphological heterogeneity, as demonstrated by the two cases described below.

Case selection, material and methods

Patient 1 was a 61-year-old male who presented with abdominal pain and intestinal hemorrhage of unknown origin. Endoscopy showed a 4 cm polypoid exulcerated lesion in the transverse colon, and abdominal CT demonstrated a mass lesion compatible with CRC stage cT3 with one suspicious node. The patient underwent hemicolectomy without postoperative complications and was referred to chemotherapy.

Patient 2 was a 41-year-old male who presented with distended abdomen and rectal bleeding. He was diagnosed with massive ascites, and a pelvine lesion of 8 cm was seen in an abdominal CT. Endoscopy showed an ulcerated rectal tumor from which biopsies were taken. Further investigations (MRT, PET) demonstrated metastatic disease in lung and liver. Because of progressive disease, the patient was referred to chemotherapy.

Formalin-fixed and paraffin-embedded blocks of the hemicolectomy tumor specimen (case 1) and endoscopic biopsies, respectively (case 2), were sectioned and stained for HE. Immunohistochemistry was performed on a Ventana Benchmark automated immunostainer (Ventana Medical Systems Inc., Tucson, AZ, USA). The antibodies used were CDX2 (Zytomed 1:50), CK20 (DAKO 1:100), CK7 (DAKO 1:1000), E-Cadherin (DAKO 1:100), p53 (Novocastra 1:100), MLH1, MSH2, MSH 6 (all Zytomed 1:20), and PMS 2 (Zytomed 1:100).

To enable separate processing of the different tumor components for molecular studies, laser microdissection was performed on 10 μm HE-stained paraffin sections under 400× magnification (System PALM/ZEISS/Carl Zeiss MicroImaging GmbH, Göttingen, Germany). DNA was extracted by proteinase K digestion overnight at 56 °C. Allele-specific real-time PCR using a mutation-specific kit (k-ras codon 12/13 CE MolBiol/TIB MOLBIOL, Syntheselabor GmbH, Berlin, Germany) and subsequent melting curve analysis was performed in duplicate on a LightCycler® (Roche Diagnostics GmbH, Mannheim, Germany). In addition, amplified DNA was bidirectionally sequenced from codon 1 through 96.

Results

Case 1

The hemicolectomy specimen showed a centrally located, ulcerated lesion 4 cm in diameter with elevated polypoid margins. Histologically, a major part of the peripheral lesion was composed of an adenoma of low nuclear grade and adjacent a small area of adenoma with high nuclear grade and development into conventional adenocarcinoma of colorectal type grade 2 WHO. Immunohistochemistry revealed expression of p53 in the invasive cancer and high grade adenoma, and showed no evidence for defects of mismatch repair proteins. The areas of adenoma low grade, adenoma high grade, invasive carcinoma, and lymph node metastasis were separately laser-microdissected. The identical results of real-time PCR and sequencing revealed a G12D mutation in the low-grade adenoma part, whereas the high-grade adenoma, the carcinoma, and the lymph node metastasis contained a G12V mutation (Fig. 1).

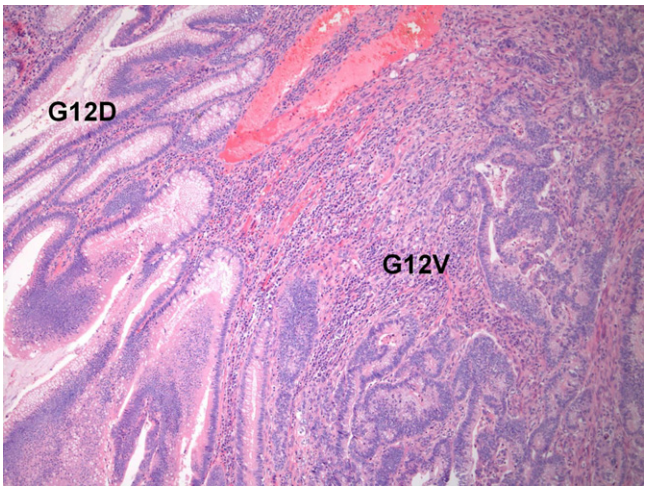

Fig. 1. Patient 1 with *K-RAS* G12D mutation in the low-grade colorectal adenoma and G12V mutation in neighboring carcinoma. H&E, 100×.

Case 2

Two-thirds of the biopsies contained a conventional glandular adenocarcinoma of intestinal type grade 2, whereas the remaining third of the biopsies was infiltrated by sharply demarcated formations of a signet ring cell carcinoma. Immunohistochemistry in both areas was identical, with positive staining for CK 20, CDX2 and E-cadherin, and negativity for CK7. No mismatch repair protein deficiency was detected.

The distinct tumor components were separated by laser microdissection. Mutation analysis concordantly showed a G12D *K-RAS* mutation in the areas of glandular adenocarcinoma, and a wild-type in the areas of signet-ring cell carcinoma (Figs. 2 and 3). Though applying allele-specific PCR, a mutated clone could not be verified in this fraction of the neoplastic cells.

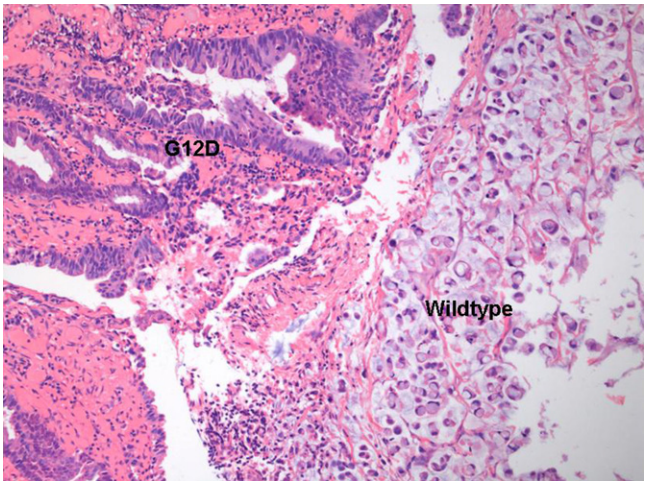

Fig. 2. Patient 2 with G12D mutation in glandular adenocarcinoma and *K-RAS* wild-type in signet ring cell carcinoma. H&E, 200×.