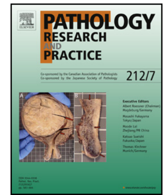
Pathology – Research and Practice 212 (2016) 598–603

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.com/locate/prp



Original article

Locally advanced rectal cancers with simultaneous occurrence of *KRAS* mutation and high VEGF expression show invasive characteristics

Milena Krajnović [a,*], Bojana Marković [a], Slavica Knežević-Ušaj [b], Ivan Nikolić [b], Maja Stanojević [c], Valentina Nikolić [c], Marina Šiljić [c], Snežana Jovanović Ćupić [a], Bogomir Dimitrijević [a]

[a] *University of Belgrade-Vinča Institute of Nuclear Sciences, 11001 Belgrade, Serbia*
[b] *Medical Faculty of Novi Sad, Oncology Institute of Vojvodina, 21204 Sremska Kamenica, Serbia*
[c] *University of Belgrade, Faculty of Medicine, Institute of Microbiology and Immunology, 11000 Belgrade, Serbia*

ARTICLE INFO

Article history:
Received 9 March 2015
Received in revised form
28 December 2015
Accepted 15 February 2016

Keywords:
Rectal cancer
KRAS
VEGF
Biomarkers
Prognosis

ABSTRACT

In this study, we investigated the mutation status of *KRAS* gene in pretherapeutic and preoperative biopsies in 63 specimens of locally advanced rectal cancers in order to evaluate its potential predictive and/or prognostic role. Regions of interest of *KRAS* exon 2 were amplified and visualized on 2% agarose gel. Obtained PCR products were subjected to direct sequencing. *KRAS* mutations were detected in 35% of patients, 91% of which were located in codon 12 and 9% in codon 13. In general, *KRAS* mutation status did not affect the response to neoadjuvant chemoradiotherapy (CRT). However, patients harboring mutated *KRAS* gene, simultaneously with high vascular endothelial growth factor (VEGF) expression, exhibited a worse response to CRT ($p = 0.030$), a more frequent appearance of local recurrences and distant metastasis ($p = 0.003$), and shorter overall survival ($p = 0.001$) compared to all others. On the contrary, patients with GGT>GCT *KRAS* mutation exhibited a significantly better response to CRT than those with any other type of *KRAS* mutation ($p = 0.017$). Moreover, the presence of GGT>GCT mutation was associated with low VEGF and Ki67 expression ($p = 0.012$ in both cases), parameters related to less aggressiveness of the disease. Our results suggest that *KRAS* mutation status could have some predictive and prognostic importance in rectal cancer when analyzed together with other parameters, such as VEGF and Ki67 expression. In addition, it seems that not only the presence but the type of *KRAS* mutation is important for examining its impact on CRT response.

© 2016 Elsevier GmbH. All rights reserved.

1. Introduction

Colorectal cancer (CRC) is the third most common cause of cancer-related death, and approximately 28% of CRC diagnoses represent rectal cancer [1]. So far, prognostic and predictive molecular markers were usually evaluated in studies, including both colon and rectal cancer patients [2,3]. Nowadays, it is evident that these tumors represent distinct clinical entities with different molecular and genetic changes [4,5]. The current management of locally advanced rectal cancer involves neoadjuvant chemoradiotherapy (CRT) followed by total mesorectal excision (TME) [6]. Despite improved local control rate, overall survival and disease-free survival [7,8], individual patient response to CRT is variable [9]. Pathological complete response is achieved in up to 30% of patients [10,11], while others do not respond or show incomplete response to the CRT [7,8]. These data may reflect heterogeneous biological properties of rectal cancer, so there is utility to define reliable biomarkers of response and personalized therapeutic approach [7,12].

Early steps of colorectal carcinogenesis involve *KRAS* oncogene-activated mutations [13]. As member of RAS/RAF/MAPK pathway, this proto-oncogene plays an important role in cell growth and proliferation [14]. *KRAS* is a membrane-bound G protein whose kinase activity is increased in approximately 30–40% of CRC by activating mutations [15]. The most common mutations occur at codons 12 and 13 of exon 2 of the *KRAS* gene, which encodes glycine [16]. Many studies have demonstrated a predictive value of *KRAS* mutation status in response of metastatic CRC patients to epidermal growth factor receptor (EGFR) inhibitors [17,18]. However, correlation between mutations of *KRAS* and clinical

* Corresponding author at: Laboratory for Radiobiology and Molecular Genetics, University of Belgrade-Vinča Institute of Nuclear Sciences, PO Box 522, 11001 Belgrade, Serbia.
E-mail address: lena2mkd@yahoo.com (M. Krajnović).

http://dx.doi.org/10.1016/j.prp.2016.02.018
0344-0338/© 2016 Elsevier GmbH. All rights reserved.

outcome in patients with locally advanced rectal cancers is not fully established. In addition, the predictive value of the immunohistochemically detected expression of various genes related to CRC was investigated. Vascular endothelial growth factor (VEGF) and Ki67, among other biomarkers, have been closely examined, but with contradictory results [19].

Hence, the aim of our study was to investigate the relationship between *KRAS* mutation status and clinicopathological and immunohistochemical features, including response to preoperative CRT and survival data, in the group of 63 locally advanced rectal cancer patients from Serbia.

2. Patients and methods

2.1. Patients and tumor samples

The study included 63 patients (40 males, 23 females; median age 64 years, range 49–82 years) with locally advanced rectal cancer (clinical stage cT3b,cN0 with positive circumferential margin (CRM) and cT4N+) who were diagnosed and treated with preoperative CRT at the Oncology Institute of Vojvodina, Sremska Kamenica, Serbia in the period 2006–2010, according to the National guidelines of the Ministry of Health of Serbia for Diagnosis, Therapy and Management of colorectal cancer). Biopsy specimens were collected during colonoscopic examination before preoperative treatment, which consisted of total irradiation dose of 50.4 Gy in 28 fractions of 1.8 Gy with concomitant application of 5-fluorouracil (425 mg/m^2) and leucovorin (25 mg/m^2). Eight to ten weeks after the completion of preoperative CRT, standardized radical surgery was performed, including TME. Location of the tumor was determined by MRI in the low (≤7 cm from anal verge), high (>7 cm from anal verge) or in the mid rectum (<7 cm and >7 cm from anal verge). The data according to all clinicopathological parameters, response to the treatment and overall survival, were taken from the medical documentation of the above mentioned institution. Immunohistochemical analysis of VEGF and Ki67 expression was performed in the preoperative biopsy specimens and was determined at the Oncology Institute of Vojvodina, Sremska Kamenica. The criterion for a positive immune reaction was a dark-brown cytoplasmic precipitate. The scoring of the staining was assessed quantitatively by counting the percentage of positive cells in 100 malignant cells at 40 total magnifications for at least 3 representative fields. We performed automatized staining by Ventana Benchmark GX immunostainer. The avidin-biotin complex (ABC) method was used for immunohistochemical detection of 0.2 mL (Clone VG1, Code M7273, LOT 00028659, Dako Denmark A/S Produktions 42 DK-2600 Glostrup) as primary antibody for the detection of VEGF protein (Kit K5204, Dako Co.). Staining kit, Code K0673, from Dako Co. has been used. According to the percentage of tumor cells in given specimens with positive immunohistochemical (IH) reaction, tumor samples were considered to have absent VEGF expression (0% of tumor cells with positive IH reaction), weak VEGF expression (1–10% of tumor cells with positive IH reaction), and moderate (10.1–50% of tumor cells with positive IH reaction) or high VEGF expression (50.1–100% of tumor cells with positive IH reaction). The same criterion was used for Ki67 expression. Response to neoadjuvant CRT was classified as positive when complete or partial remission (CR/PR) was detected or negative, in the case of the presence of stable or progressive disease (SD/PD). All procedures were carried out with the prior informed consent of the patients and with the approval of the local Ethics Committee.

2.2. DNA extraction and mutation analysis

Formalin-fixed, paraffin-embedded tumor tissues obtained prior to the start of radiochemotherapy were used for all analyses presented here. Genomic DNA was isolated from deparaffinized tumor specimens using standard proteinase K, phenol/chloroform/isoamyl alcohol extraction, and ethanol precipitation [20]. Exon 2 of *KRAS* gene, containing codons 12 and 13, was amplified with primer sets described earlier [21]. The PCR mixture contained 1× Gold Buffer (Applied Biosystem, Foster City, CA, USA), 1.5 mmol/L $MgCl_2$, dNTP (each at 0.2 mmol/L), primers (0.2 μmol/L each per reaction) and 0.625 units of AmpliTaq Gold® DNA Polymerase (Applied Biosystems, USA) in a final volume of 25 μL. Amplification was carried out in an Applied Biosystems 2720 temperature cycler by initial denaturation at 95 °C for 3 min, followed by 40 cycles (30 s at 94 °C, 30 s at the annealing temperature being specific for each reaction [21], and 30 s at 72 °C, followed by final extension for 10 min at 72 °C). PCR products were visualized on 2% agarose gel, followed by purification with Qiagen Minelute PCR purification kit (Qiagen, Hilden, Germany). Cycle sequencing reactions for both genes were performed with 2 μL of each purified product using the BigDye Terminator system v3.1 ready reaction kit (Applied Biosystems, Foster City, CA, USA) and primer pairs as described earlier [21]. Sequenced products were further processed with an automated ABI Prism 310 Genetic Analyzer (Applied Biosystem, Foster City, CA, USA), at the Institute of Microbiology and Immunology, University of Belgrade Faculty of Medicine, Belgrade, Serbia.

Table 1
Types of *KRAS* mutations within the samples of rectal cancer.

Codon (wt sequence)	Nucleotide exchange	Amino acid exchange	Number
12 (GGT)	GAT	G12D	6/22 (27%)
	GTT	G12V	8/22 (36%)
	GCT	G12A	4/22 (18%)
	TGT	G12C	1/22 (5%)
	AGT	G12S	1/22 (5%)
13 (GGC)	GAC	G13D	2/22 (9%)

Wt, wild type.

2.3. Statistical analysis

Contingency tables were analyzed using Pearson's χ^2-test or Fisher's exact two-tailed test when expected frequencies were lower than five. Continuous variables were compared with the use of Student's *t*-test. Overall survival distributions were estimated by the Kaplan–Meier method, and differences were evaluated by the Log-rank test. In all tests, a *p* value less than 0.05 was considered statistically significant. All statistical analyses were performed using the Sigma Plot 10.0 licensed statistical analysis software package.

3. Results

3.1. KRAS mutation status

Analysis of *KRAS* mutation status was successfully performed in all 63 cases. In general, *KRAS* mutations were detected in 35% (22/63) of patients, 91% (20/22) of which were located in codon 12 and 9% (2/22) in codon 13. The spectrum of observed *KRAS* mutations is shown in Table 1. The most commonly observed mutation was GGT>GTT transversion at the second position of codon 12, resulting in a glycine to valine substitution (G12V). This type of *KRAS* mutation was detected in 36% (8/22) of cases. According to the type of base pair exchange, distribution of detected mutations was as follow: 41% (9/22) of G>T transversions, 41% (9/22) of G>A transitions and 18% (4/22) of G>C transversions. Not one patient exhibited more than one type of *KRAS* mutation.

Download English Version:

https://daneshyari.com/en/article/2155063

Download Persian Version:

https://daneshyari.com/article/2155063

Daneshyari.com