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Case Report

Coexistence of G12V and K117N Substitutions in KRAS Gene with E542K Substitution in PIK3CA Gene in Rectum Adenocarcinoma

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Abstract

Colorectal Cancer (CRC) is one of the leading causes of cancer mortality worldwide. In the last decade, median overall survival has increased significantly with the introduction of new cytostatics and molecularly targeted therapies. Notably, the definition of molecular profile could predict effectiveness of different treatment options. Evaluation of the mutational status of KRAS, NRAS, and BRAF genes is a crucial step in qualification of CRC patients for the proper treatment regimen. In the present study, we report an additional case of a metastatic rectum adenocarcinoma showing co-existence of three somatic mutations-G12V and K117N in the KRAS gene and E542K in PIK3CA gene. In spite of the mentioned intratumoral heterogeneity the patient benefited from first-line FOLFIRI chemotherapy.

Keywords: Colorectal cancer; Intratumoral heterogeneity; KRAS; PIK3CA

Abbreviations

ASP-q PCR: Allele-Specific Quantitative Polymerase Chain Reaction; CRC: Colorectal Cancer; CT: Computed Tomography; HRM-PCR: High-Resolution-Melting Polymerase Chain Reaction; OS: Overall Survival; PFS: Progression-Free Survival; wt: Wild-Type

Introduction

Colorectal Cancer (CRC) is the third most commonly diagnosed cancer in males and the second in females, however, the CRC survival rate is not substantially associated with gender. It was also reported that better prognosis for 5-year survival was indicated both in the local stages of CRC and in younger patients [1]. 70-80% of newly diagnosed CRC patients have localized disease and can be treated with surgical resection and adjuvant chemotherapy with cytotoxic agents [1,2]. However, remaining patients can be treated with chemotherapy, optionally with the addition of anti-EGFR antibodies (cetuximab, panitumumab). Absence of mutations in RAS genes is a precondition for the effectiveness of molecularly targeted therapies. Therefore, evaluation of the molecular bio-markers in tumor cells may play crucial role in predicting the response to targeted drugs in CRC [3]. It was previously indicated that KRAS, BRAF, NRAS and PIK3CA mutations have a negative impact on the response to EGFR inhibitors [3,4,5].

Previous studies have also noted that 5-10% of primary CRC tumors demonstrate an intratumoral heterogeneity of KRAS, BRAF, and PIK3CA mutations. The differences in the type of KRAS mutations or the co-existence, these mutations with other gene mutations between primary and metastatic tumors can prove the theory of neoplastic heterogeneity [3,6,7]. However, the detection possibility of two or more mutations in the same codon has seldom been reported in CRC and was limited to KRAS gene. Moreover, the clinical impact of multiple mutations on patient prognosis has not yet been well studied and clarified [8,9].

In the following study, we reported a case of the coexistence of two KRAS somatic mutations (G12V and K117N) with the E542K substitution in exon 9 of PIK3CA in tumor cells of male patient affected by an advanced rectum adenocarcinoma. Both NRAS and BRAF gene mutations were not detected. DNA from formalin-fixed, paraffin embedded tumor tissue was isolated using QIAamp DNA FFPE Tissue Kit (Qiagen, Canada). Analysis of KRAS, BRAF and NRAS mutations was performed using the Entrogen Mutation Analysis Kit (EntroGen, USA) with allele-specific, fluorescent and hydrolysis molecular probes in Cobas z480 real-time device (Roche Diagnostic, USA). PIK3CA mutations were analyzed using two techniques in Eco Illumina real-time device (Illumina, USA). The HRM-PCR (high-resolution-melting) was performed as by pre-screening method to select potential positive samples and ASP-qPCR (allele-specific quantitative PCR) was used to identify the type of PIK3CA mutations (E542K, E545K, and H1047R).

Case Report

At the beginning of 2014, a 70-year-old Caucasian male patient was admitted to a Gastroenterology Department at the Provincial Specialist Hospital in Lodzdue to weight loss (10kg within 6months), abdominal pain, and loose stools with macroscopic blood. In his previous medical history prostate hypertrophy (diagnosed 5years-ago) were noted. Physical examination and gastroscopy were identified as normal. However, colonoscopy showed a rectal polypus-like tumor (7-12cm from the sphincter) and abdomen ultrasound examination revealed multiple liver lesions that could suggest corresponding metastases. A polypus biopsy supported the diagnosis of invasive rectum adenocarcinoma (grade-2).

The Computed Tomography (CT) scans allowed identifying the

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primary rectum tumor (20x55mm-starting from sphincter (40mm) and infiltrating adipose tissue). The liver metastases (90x93x77mm) as well as multiple metastatic lesions in both lung and in right adrenal gland. Increased levels of CEA (129.2 ng/ml), CA19-9 (225,3 U/ml) and alkaline phosphatase (252 U/L) were detected.

Because of intestine obstruction the patient was directed to the surgical department at Provincial Specialist Hospital in Lodz, where explorative laparotomy and palliative sigmoidostomy were performed without further complications. In the next step the patient was referred to the Regional Center of Oncology in Lodz for further treatment. In May 2014, he has started chemotherapy, according to FOLFIRI schedule and during treatment we observed rapid decreasing of CEA level. Controlled CT scan performed in August 2014 confirmed regression of all metastatic lesions according to RECIST 1.1 criteria. The patient continued first-line chemotherapy with any toxicity and signs of progression. In May 2015, stable disease was described.

Discussion

Genetic instability in cancer cells can be demonstrated in the presence of a single or multiple mutations and indifferences in the molecular profile of primary and metastatic lesions. Such mutational heterogeneity is reported in 5-10 % of CRC cases and can affect both on chemo- and radio-sensitivity [6,7,8]. Various mutations can differ in carcinogenic potential that explains differences in overall survival (OS) of patients with heterogeneous primary CRC. The majority of co-mutations affect only one gene or codon, however co-mutations also may be observed between various genes and codons [8,9].

Previous studies have demonstrated that KRAS mutations can both predict resistance to anti-EGFR therapy and increase the risk of recurrence or mortality in patients with CRC [4,5,10]. Moreover, Smith et al. Indicated that KRAS mutations at codon 12 are associated with stronger transforming activity in vitro than mutant variants of codons 13, 61, 117 and 146 [4,11,12]. Maughan et al. also reported strong prognostic effect of BRAF and NRAS genes, mutations in CRC patients [13]. Initial reports showed that all PIK3CA mutations (both in exon 9 and 20) can be associated with impaired response to cetuximab and chemotherapy, however, some studies indicated the different effects of various types of PIK3CA mutations as the markers of sensitivity to anti-EGFR monoclonal antibodies [3,4].

In the following report we have described a patient with invasive rectum adenocarcinoma who had identified the coexistence of three mutations in KRAS and PIK3CA genes that have not been previously described in the literature. Improta et al. Described the coexistence of two KRAS gene mutations that were located in the same codon (G12D and G12V). The mutations were identified in a female patient affected by an advanced rectum adenocarcinoma. The patient was treated by chemotherapy before and after surgery without no side effects and complications. However, the liver metastases were identified early [8]. Foltran et al. have observed that KRAS, BRAF and NRAS mutations were mutually exclusive in their series of 194 metastatic CRC. 11.9 % of the samples carried both KRAS and PIK3CA mutations. They also noted that longer OS was associated with resection of the primary tumor and wild-type by examining genes [4]. Mao et al. Noted coexistence of KRAS and BRAF mutations in 24 % out of 69 analyzed CRC patients. Furthermore, in 12% of analyzed samples harbored both KRAS and PIK3CA mutations [14].

Unexpectedly, in our analysis, we observed good response for the first-line chemotherapy according to FOLFIRI schedule. Liao et al. and Shen et al. noted that PIK3CA mutations (especially in exon 9) shared a strong association with KRAS mutations causing negative prognosis with shorter median progression-free survival (PFS) in Asian population [15,16]. However, European study performed by De Rock et al. indicated than only PIK3CA mutation located in exon 20 are associated with worse clinical outcome [17]. According to these data the presence of K117N substitution in KRAS gene and E542K in exon 9 of PIK3CA gene could hide the aggressive activity of G12V mutation in KRAS gene.

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