Correlations Between Distinct Ras Mutations and Response to Bevacizumab in Patients with Colo-rectal Cancer

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CRC is currently ranked third among frequently diagnosed malignancies. Mutations in exons 2,3 and 4 of the KRAS and NRAS genes were detected using a targeted resequencingassay from paraffin embedded tissue from patients' biopsies samples. The following data was recorded for each patient: baseline information - age and sex – as well as diagnosis, cancer localization, disease stage and laboratory parameters.In our study group we could not find statistically significant differences regarding distinct mutations and the progression free survival ($p=0.34>0.05$) for patients treated with an anti-angiogenic therapy.

Keywords: colorectal cancer, RAS, mutations, bevacizumab.

Colorectal cancer (CRC) is the fourth cause of neoplasm-related mortality worldwide with an increasing incidence and a prediction of more than 2.2 million new cases by 2030 therefore amplifying its burden by 60% and resulting in 1.1 million cancer deaths. CRC is currently ranked third among frequently diagnosed malignancies [1-3].

Angiogenesis plays a key role in tumor growth, invasion and metastasis. Solid tumors reach a maximum size of 1-2 mm in the absence of adequate vascular supply. Beyond that extent, critical nutrients and oxygen can only be provided by means of angiogenesis [4].

The vascular endothelial growth factor (VEGF) acts as an angiogenic agent in tumors and is associated with vascular invasion[4]. It acts predominantly on the vascular endothelial cells and stimulates the development of new blood vessels.[5] VEGF expression in normal colonic mucosa and adenomas is minimal to absent, whereas its expression is reported in approximately 50% of CRCs[6].

The formation of new blood vessels from pre-existing vasculature is a complex process which has prompted extensive research in order to develop novel anticancer therapies ever since Folkman first introduced anti-angiogenesis as a potential strategy in 1971 [7].

Bevacizumab is a humanized monoclonal antibody that binds to VEGF and was the first agent to target the angiogenetic mechanism[8].Bevacizumab binds VEGF and prevents the interaction of VEGF to its receptors (Flt-1 and KDR) on the surface of endothelial cells. The connection between VEGF and its receptors leads to endothelial cell proliferation and new blood vessel formation in *in vitro* models of angiogenesis[9].

It is believed that VEGF up-regulation follows oncogenic mutations or amplification of RAS. RAS gene family (KRAS, NRAS, and HRAS) encode p21 proteins which are small guanine-nucleotide binding proteins with important roles in several signal-transduction pathways [10].

Mutant RAS results in a lasting growth-promoting signal and is present in 40% of adenocarcinomas of the colon, with mutations in codon 12 by far the most frequent [11]. Codon 12 RAS mutations have different carcinogenic potential and prognostic. Studies associate KRAS G12V with a greater mortality and more aggressive neoplasia than other codon 12 mutations[12] including G12D - the most common –due to its structure [10].

Experimental part

Participants and methods

Patients treated for colorectal cancer at the Medical Oncology Departament at Oncolab Craiova and the Municipal Hospital Filantropia Craiova, Romania, over a period of 9 years (between May 2010 and May 2019), were included in our study.

Inclusion criteria were age >18 years, a histologically confirmed adenocarcinoma of the colon or rectum with metastatic disease, the presence of mutations of KRAS or NRAS genes and, treatment with Bevacizumab. Patients with wild-type KRAS mutations were excluded.

The following data was recorded for each patient: baseline information - age and sex – as well as diagnosis, cancer localization and disease stage. Laboratory parameters such as complete blood cells count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), creatinine, glucose levels, carcino-embryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) values were recorded at the time of admission.

The tumoral tissue samples were gathered from 40 patients with colorectal adenocarcinoma and classified according to the pTNM staging, in line with the criteria endorsed by American Joint Committee on Cancerfor colon and rectum [13-15]. As a result, the cancer grade was divided into three stages (G1, G2, and G3).

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The molecular analysis was conducted using DNA extracted from paraffin embedded tissue from patients' biopsies samples. Mutations in exons 2,3 and 4 of the KRAS and NRAS genes were detected using a targeted resequencing assay.

In order to assess disease progression throughout treatment with bevacizumab, we have applied the criteria put forward by RECIST Guidelines Version 1.1. [16]

Statistical analysis

Normal distribution of the study data was tested.The means were expressed using central tendency indicators: means, median, standard deviation, 95% confidence interval of the means and the range. A p-value less than 0.05 was considered statistically significant.

Results and discussions

Participants

Forty patients who met the inclusion requirements and did not fulfil exclusion criteria were included in our study. The mean age of the study population was 68 ± 8.28 years (range: 41-78). The majority of the patients were men (28 men vs 12 women). All the patients were diagnosed with adenocarcinomas and more than half (57.50 %) of the tumours were classified with the G2 histopathological grading – moderately differentiated. Regarding the disease stage, 65% of participants were labelled with T3 stage. The large bowel was classified according to anatomical, clinical, and therapy characteristics resulting in 4 main locations of the neoplasia: right colon - ascending and cec, transverse colon, left colon - descending and sigmoid colon, and rectum. The most frequent diagnosis was rectal cancer (52.50 %).

In our study we evaluate different parameters of patients with colorectal cancer in order to observe if there are correlations that can be made and help to determine the prognostic role of these data. In the future, we plan on extending the group of patients and we aim to obtain information that will help with the predictive role of treating CRC with bevacizumab.

The mean laboratory parameters of the participants are summarized in table 1.

The three human *RAS* genes (*KRAS, NRAS* and *HRAS*) are the most frequently mutated oncogenes in human cancer[17] appearing in 45% of colon cancers, making *RAS* one of the most important targets in oncology for drug development [18].

KRAS mutation occurs with the greatest frequency in all human cancers (21.6%), followed by *NRAS* (8.0%)[19,20].Molecular analysis shows a predominance of KRAS mutations (90%) over the mutation of NRAS gene (10%) in our study population.

Nearly 97% of all *KRAS* mutations are localized to codons 12 or 13 [21,22] and these data are correlated with our findings: in relation to RAS genes, mutations in codon 12 are more frequent (72.22 %).

From several point mutations, our data show that the most common is codon 12 Gly→Val (G12V), present in 27.78% of cases. The data gathered thus far now on isoform-specific signalling is not yet comprehensive, although it seems to be clear that the differences are relatively refined and tissue context specific. This is exemplified in comparative studies that revealed that G12V mutated K-Ras promotes endodermal stem cell expansion by promoting proliferation and inhibiting differentiation [23].

	Right Colon	Transverse colon	Left colon	Rectum
Leucocytes/mm³	6818.57	8500	7704	8795.50
Hemoglobin g/dl	11.71	11.9	12.1	12.51
Platelets/mm³	297.57	390.50	245.30	272.65
CA19-9 U/L	83.79	28.10	31.04	7753.77
CEA ng/mL	53.41	108.88	291.32	655.18
AST U/l	35.87	17.61	25.61	23.29
ALT U/l	53.87	17.45	21.41	19.08
GGT U/l	138.14	152.00	66.09	77.10
ALP U/l	371	133.00	197.70	146.58
Creatinine mg/dl	0.92	0.85	0.91	2.93
Glucose mg/dl	94.85	104.11	90.73	111.62

Table 1
CORRELATIONS BETWEEN MEAN LABORATORY PARAMETERS AND LOCALIZATION OF COLORECTAL CANCER

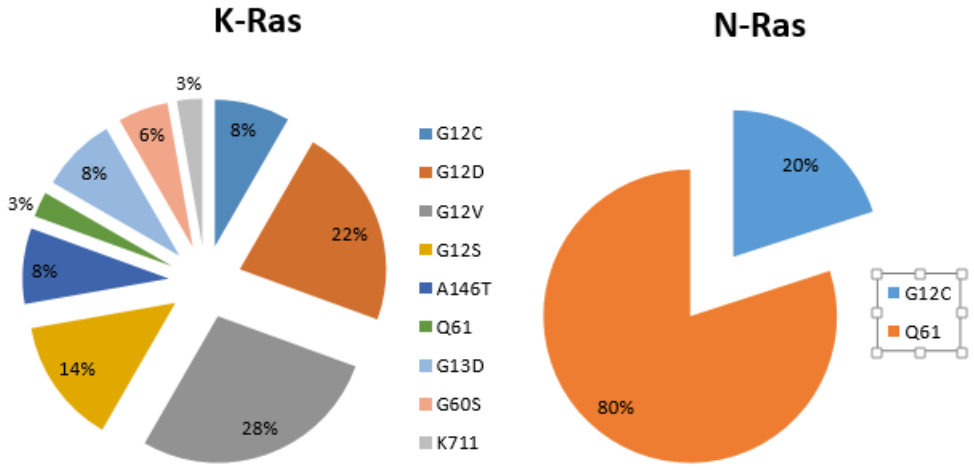

Table 2
POINT MUTATIONS IN KRAS AND NRAS GENES

	Right colon	Transverse colon	Left colon	Rectum
PFS	396±184	380.5±30.7	336.2±107	422±95

Table 3
CORRELATION BETWEEN TUMOUR LOCALIZATION AND PROGRESSION-FREE SURVIVAL (PFS)

Table 4
ASSOCIATION BETWEEN RAS MUTATION AND PROGRESSION-FREE SURVIVAL (PFS)

Mutation point	G12C	G12D	G12V	G12S	A146T	Q61	G13D	G60S	K117
Average PFS	320	420	486	188	425	531	290	380	548

The patient group treated with bevacizumab and diagnosed with rectal cancer displayed on average the longest progression-free survival period (422 ± 95 days). However, based on the result of the ANOVA test (p=0.77>0.05) the differences between PFS are not statistically significant.

The participants with KRAS mutations in codon 12 (G12S) averaged a lesser PFS interval, suggesting a more aggressive tumor phenotype. However, the data is not sufficient for the p value to go below the threshold that indicates statistical significance (p=0.34>0.05) yet other studies state that*KRAS* codon 12 mutations, and specifically G12V and G12S mutations, are associated with worse prognosis after resection of colorectal liver metastases andthese mutations can be considered independent prognostic factors [24].

Conclusions

Our research showed that there is a great heterogeneity in ras mutated colo-rectal carcinoma. In our study we could not find statistically significant differences regardingdistinct mutations and the progression free survival (p=0.34>0.05) for patients treated with an anti-angiogenic therapy.

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