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Journal of Gastroenterology Research and Practice

Research Article

Open Access, Volume 4

Molecular characteristics of left-sided, advanced stage colorectal cancer: A Mexican perspective

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Email: deandajaz@hotmail.com

Received: Feb 13, 2024

Accepted: Mar 21, 2024

Published: Mar 28, 2024

Archived: www.jjgastro.com

Keywords: Advanced colorectal cancer; KRAS; NRAS; BRAF; MSI.

Abstract

Background: Predictive molecular markers based on the consensus molecular classification of colorectal carcinoma are tested following expert recommendations. Specifically, they are tested when target molecular therapy is being considered for treatment. Their presence depends on biology, but their frequency seems to be increased in advanced stages.

Objective: We developed a study of cases of left-sided CRC in advanced clinical stages from a cohort of Mexican patients to evaluate the presence of predictive molecular markers.

Methods: Predictive molecular markers were analyzed, including BRAF, KRAS, NRAS and mismatch repair genes, in cases of advanced, left-sided colorectal carcinoma.

Results: An alteration was found in more than a half of cases. KRAS was the most frequently mutated gene, in more than a third of cases. No BRAF mutation was found.

Conclusion: The main determinants of predictive molecular markers appear to be staging, location and methodology. More studies need to be done to determine the variability given by race.

Introduction

Colorectal Carcinoma (CRC) is a neoplasia with a double-edge sword. Effective secondary prevention has been key to its management. Though its mortality rate varies depending on the population studied, data shows more than 50% of patients develop metastasis [1,2]. The changing epidemiological trend towards diagnoses in younger ages is also a matter of concern.

The epidemiology of CRC is best explained by its associated risk factors. Diet, obesity, the microbiota and the immune response contribute to the metabolic alterations seen in CRC [3]. Many molecules interact in the carcinogenesis of CRC. There are molecular signatures that have been identified as being different in the sequence of events, molecular interactions and biology of CRC. The main drivers are oncogenes and tumor suppressor genes, including KRAS, BRAF, PIK3CA, APC and p53 [3]. Accordingly, a molecular classification of CRC has been developed.

Historically, the molecular classification was based on the genetic model of CRC [4]. CRC arises from a precursor lesion, a dysplastic polyp. It was believed that these clonal cells had specific genetic alterations that followed a relatively constant pathway towards carcinoma.

In recent times, the CRC Subtyping Consortium analyzed published data on CRC subtyping. Four Consensus Molecular Subtypes (CMS) were identified [5].

CMS 1 corresponds to microsatellite unstable tumors. They harbor alterations in BRAF and overexpress genes associated with inflammation. CMS2 show higher chromosomal alterations, specially at oncogenes. They upregulate CMYC and WNT pathways. CMS3 show lower chromosomal alterations, higher prevalence of CpG island methylator phenotype-low clusters. They are associated with mutations in KRAS and metabolic alterations. CMS4 is associated with epithelial-mesenchymal transition and alterations in genes associated with the biology of metastasis [6].

Based on the CMS, molecular targets are being used to treat CRC. Specifically, CRC with metastasis [7]. In 2017, the American Society for Clinical Pathology, College of American Pathologists (CAP), the Association for Molecular Pathology and the American Society of Clinical Oncology established recommendations for the molecular biomarker testing and their corresponding treatments [8]. In summary, they concluded predictive molecular markers for CRC include KRAS, NRAS, BRAF and Mismatch Repair genes (MMR).

The clinical utility of studying the molecular subtypes of CRC specimens is not debatable. Recommendations are still not strong enough, reflecting the need of robust studies analyzing predictive molecular markers in CRC. We developed a study of cases of left-sided CRC in advanced clinical stages from a cohort of Mexican patients to evaluate the clinical utility of predictive molecular markers.

Materials and methods

A retrospective cohort of cases from the Oncology Hospital, National Medical Center Siglo XXI in Mexico City was done including all cases of advanced stage, left-sided colorectal carcinoma with molecular analysis of NRAS, KRAS and BRAF, as well as immunohistochemical determination of microsatellite instability from the years 2022 to 2023. It included adult patients of both sexes. Cases without a complete biomarker analysis were excluded. The study was approved by the internal Research Ethics Committee.

DNA was extracted from formalin-fixed paraffin specimens of the primary CRC, using the Biocartis IdyllaTM System for KRAS (BCT005812), NRAS and BRAF (A0030/6) (Table 1). The system determines the presence of mutations through Real-Time Polymerase Chain Reaction (RT-PCR).

Immunohistochemistry assays for MMR were performed in the same samples, using the following antibodies: MLH1 (Mob430), PMS2 (PDM171), MSH2 (Mob585), and MSH6 (Mob429). Their results were interpreted according to the guideline from the College of American Pathologists.

The statistical analysis was performed using GraphPad Prism 10. The chi-squared test was used to compare categorical groups and Mann-Whitney test was used for continuous variables. A p value of <0.05 (two-tailed) was considered significant in all the statistical tests.

Results

A total of 98 cases were included. Cases with inadequate tissue were eliminated. The median age was of 63(58-66). The male-to-female ratio was of 1.64. A total of 58(59%) cases had an abnormal predictive molecular marker. The most frequently mutated marker was KRAS (see Figure 1a). There were only 5 cases with mutations in NRAS, and 4 cases with altered MMR status. There were no cases with mutations in BRAF.

The most prevalent mutation in KRAS was in codon 12 (Figure 1b). The number of cases with mutations in NRAS were too small to determine a prevalence. Cases with altered MMR were of two profiles: Loss of MLH1 only or loss of MLH1 paired with PMS2. None of these cases presented mutation in BRAF. Analysis of the age differences in the cases with KRAS mutation, NRAS mutation and deficient MMR yielded no information. However, the case numbers for the latter too are small.

Table 1: Genes, codons and mutations analyzed.

Gene	Number of mutations	Exon	Codons	Mutations	
KRAS/NRAS	21	2	12	G12C	(c.34G>T)
				G12R	(c.34G>C)
				G12S	(c.34G>A)
				G12A	(c.35G>C)
				G12D	(c.35G>A)
				G12V	(c.35G>T)
			13	G13D	(c.38G>A)
		3	59	A59E	(c.176C>A)
				A59G	(c.176C>G)
				A59T	(c.175G>A)
			61	Q61K	(c.181C>A; c.180_181delinsAA)
				Q61L	(c.182A>T)
				Q61R	(c.182A>G)
				Q61H	(c.183A>C; c.183A>T)

		4	117	K117N	(c.351A>C; c.351A>T)
			146	A146P	(c.436G>C)
				A146T	(c.436G>A)
				A146V	(c.437C>T)
BRAF	5		600	V600E	(c.1799T>A; c.1799_1800delinsAA)
				V600D	(c.1799_1800delinsAC)
				V600K	(c.1798_1799delinsAA)
				V600R	(c.1798_1799delinsAG)

Table 2: Results of the systematic analysis.

Year	Anthors	Method	Exons	Total n	n	Stage	Age	M.F	Laterality	KRAS %	NRAS %	BRAF %
2013	Rosty et al..	KRAS-RT PCR BRAF allele specific PCR	KRAS exon 2, BRAF V600E	776		all	6818	1.09	all	28.00	n/a	16.00
					295	advanced			all	29.10	n/a	19.60
					210	all			right	32.30	n/a	30.40
					63	all			transverse	28.50	n/a	20.60
					463	all			left	26.70	n/a	9.20
2015	Mans et al.	RT-PCR; dye terminator sequencing for BRAF	KRAS exon 2	431		advanced	61 (27-92)	1.37	all	42.00	n/a	8.00
					161	advanced			right	48.40	n/a	15.00
					239	advanced			left	42.60	n/a	4.60
2016	Nam et al.	RT-PCR	KRAS codons 12, 13, 61; BRAF V600E	191		all	60 (28-93)	1.17	all	54.40	n/a	3.10
					170	advanced			all	56.40		3.50
					49	all			right	71.40	n/a	8.20
					142	all			left	48.50	n/a	1.40
2016	Sharma et al.	RT-PCR	KRAS exon 2	461		advanced	61 (26-89)	1.17	all	37.30	n/a	n/a
2017	Lee et al.	RT-PCR	KRAS codon 12, 13,61	262		advanced	62 (32-93)	1.56	all	46.60	n/a	n/a
2017	Hua Gae et al.	RT-PCR	KRAS exon 2,3,4; BRAF exon 15	289		all	59.6	1.8	all	42.70	n/a	2.30
					43	all			right	37.20	n/a	7.00
					179	all			left	43.50	n/a	1.10
2019	Franczak et al.	RT-PCR	RAS exon 2,3,4; BRAF exon 15	50		advanced			all	44.00	7.00	11.00
2019	Wojas- Krawc-zyk et al.	RT-PCR	RAS exon 2,3,4; BRAF exon 15	102		advanced	64+/- 9.41	2.09	all	30.30	3.90	6.87
					32	advanced			right (A,T,D)	19.00	6.25	6.25
					63	advanced			left	32.00	1.50	8.00
2020	Van Tactal	RT-PCR	RAS codon 12, 13, 61; BRAF exon 15	156	79	advanced	59.5	1.1	all	44.00	14.00	10.00
2020	Bourhis et al.	RT-PCR	RAS codon 12, 13, 61; BRAF exon 15	20		advanced	45-90	0.87		55.00	5.00	15.00
2021	Alharbi et al.	RT-PCR	RAS codon 12, 13, 61; BRAF exon 15	248		all	63 1 14	1.62	all	50.00	2.00	0.40
					65				right	65.00		
					117				left	45.00		
2021	Delatkhah et al.	RT-PCR	RAS	173		advanced	58 + 12.95	1.27	all	44.00	1.30	2.50
					20				right	35.00	n/a	n/a

					87				left	44.00	n/a	n/a
2022	Makutani et al.	RT-PCR	RAS codon 12, 13, 61; BRAF exon 15	253		all	72 (32- 92)	1.11	all	44.00	3.60	18.00
2022	Alghamdi et al.	RT-PCR	KRAS exon 2,3,4	194		all	58 1 13	1.12	all	50.00	n/a	n/a
					47				right	76.00		
					135				left	45.00		
2022	Bezyk et al.	RT-PCR	RAS exon 2,3,4; BRAF exon 15	500		advanced	66	1.5	all	38.00	4.00	4.80
					89	advanced			right	52.00	3.40	10.00
					17	advanced			transverse	47.00	0.00	23.50
					337	advanced			left	32.60	4.70	2.70
2023	Mahdi et al.	RT-PCR	RAS exon 2,3,4	414		advanced	59 + 16	1.08	all	52.00	3.00	n/a
				105					right	60.00	2.00	n/a
				299					left	50.00	3.40	n/a
2023	Radanova et al.	RT-PCR	KRAS exon 2,3,4	236		advanced	63.92 + 10.52	1.4	all	47.00	n/a	n/a
					70	advanced			right	54.20	n/a	n/a
					166	advanced			left	44.00	n/a	n/a
	Present study	RT-PCR	RAS exon 2,3,4; BRAF exon 15	98		advanced	59	1.64	left	49.00	5.00	0.00

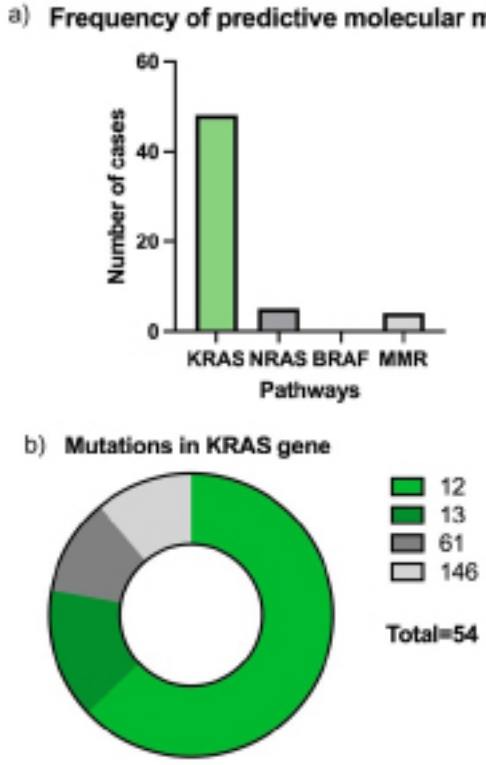

Figure 1: Graphical representation of **(a)** the frequency of predictive molecular markers and **(b)** the frequency of mutations in each foci analyzed in KRAS.

To identify differences in the frequency of mutations, a search was done in PUBMED using the terms "advanced colorectal", "KRAS" and "PCR". The search yielded 117 results, of which only 17 were considered for the final analysis; only RT-PCR base assays were considered. The data retrieved includes the results for KRAS, NRAS and BRAF mutation for advanced CRC (stage III and IV; mCRC), left-sided CRC (descending colon, sigmoid and rectum), transverse CRC, and right-sided CRC (ileocecal valve, cecum, and ascending colon). They are enlisted in Table 2 [9-25].

Discussion

Predictive molecular markers for CRC include KRAS, NRAS, BRAF and mismatch repair genes. The American Society for Clinical Pathology, CAP, the Association for Molecular Pathology and the American Society of Clinical Oncology have established recommendations based on expert opinion [8]. The expert consensus opinion considers formalin fixed paraffin embedded tissue acceptable for molecular analysis. Both metastatic and recurrent are the preferred CRC tissues for testing.

The mutation status of CRC can be tested through direct Sanger sequencing, PCR or pyrosequencing. The DNA extraction may be done from paraffin-embedded tissue followed by dissection of the tumor. Pathology reporting protocols from the CAP recommend identifying every method used accordingly [26].

One of the best non-NGS methods for the identification of RAS-BRAF mutations in CRC is indeed PCR. Considering the Idylla platform, a French meta-analysis calculated a sensibility of 99.3% and specificity of 96.8% with a positive predictive value of 97.4% and negative predictive value of 99.1% for KRAS. Similarly, they reported a sensibility of 96.7% and 98.8%, a specificity of 99.7% and 99.3%, a PPV of 99% and 98%, with a NPP of 98.9% and 99.6% for NRAS and BRAF respectively [27].

Numerous PCR-based methods have been validated to test for predictive molecular markers. Their sensibility is higher than that of Sanger sequencing. The frequency of mutations, however, varies according to specific variables. For instance, age and sex are not associated to mutations [28,29]. Nor are the degree of differentiation, the presence of lymph vascular invasion or perineural invasion [29]. Race could be important; however, the main determinants appear to be staging, location and methodology (Table 2).

The frequency of mutations in predictive molecular markers are found in more than 50% of cases with CRC, a similar rate as found in the cohort (59%). KRAS is the most mutated gene (in 35-55% of cases as found in the literature; 49% of cases in

our cohort). This remains true independently of the population studied (Table 2).

KRAS is highly associated to right-sided CRC. Nevertheless, left-sided CRC may present alterations in KRAS, even if Microsatellite Stable (MSS). The frequency is lower but depends on the staging of the disease (Table 2). NRAS is mutated less often, and there is no association to sidedness (Table 2).

According to the CMS of CRC, right-sided CRC are commonly unstable. Left-sided CRC are less frequently associated to MMR, but not an impossibility. In our cohort of left-sided CRC, cases lacking only MLH1 may reflect methylation of MLH1, which is associated to BRAF mutations. However, none had mutation in BRAF. mCRC tumors present BRAF mutation in 5-10% of cases (Table 4). When comparing MSS, right-sided and left-sided CRC, BRAF mutations are statistically more frequent in MSS right-sided CRC [30,31]. Sidedness may thus explain our unanticipated result for BRAF.

Mutations in genes from the MAPK pathway (specifically KRAS and NRAS) not only predict resistance to tyrosine kinase inhibitors but also seem to predict survival [29,32-34]. Clinical decision-making depends on the exhaustiveness of their study. Though there is a low rate of concomitant mutations (NRAS/KRAS and KRAS/BRAF), their existence may be relevant [28,29]. The low rate may be explained by studies analyzing tumor heterogeneity in mCRC, which have identified small clones having mutations at other exons of KRAS [35,36]. Their presence may affect the effectiveness on target therapy. With this understanding, PCR-based studies have, through time, increased the number of exons being studied in both genes (KRAS and NRAS) (see Table 2).

Conclusion

In present times, RAS mutant CRC is adequately studied by PCR-based platforms. The frequency of predictive molecular markers depends on the technique used, the staging of CRC, and the location of CRC. These variables are adamant when analyzing the impact of population/race in the epidemiology of RAS mutant CRC.

Declarations

Acknowledgements: The study did not receive fundings.

Conflict of interest: The authors declare no potential conflicts of interest.

Author contributions: MGJDA provided the conceptualization, data curation, project administration and resources and review and editing; MJLT provided with methodology, formal analysis and writing of original draft; RMG and BMB provided conceptualization, investigation, supervision, review and editing; JMMN provided with conceptualization, resources, project administration, review and editing, supervision.

Availability of data and materials: The data generated in this study are available upon request from the corresponding author. Raw data for this study were generated at the Molecular Biology laboratory at the Hospital. Derived data supporting the findings of this study are available from the corresponding author upon request. All data analyzed during this study are included in this published article. The raw data generated at the core facilities may be accessed by the corresponding author upon request.

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