KRAS MUTATIONS CORRELATES WITH P53 STATUS IN COLORECTAL CARCINOMAS

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BACKGROUND

KRAS is a proto-oncogene located on chromosome 12 that encodes a small GTP-binding protein that acts as a self-inactivating signal transducer by cycling from GDP- to GTP-bound states in response to stimulation of cell surface receptors, including EGFR. KRAS oncogenic mutations, present in approximately 30-50% of colorectal carcinomas (CRC), result in a constitutively active protein and lack of response to EGFR inhibitors (Figure 1).

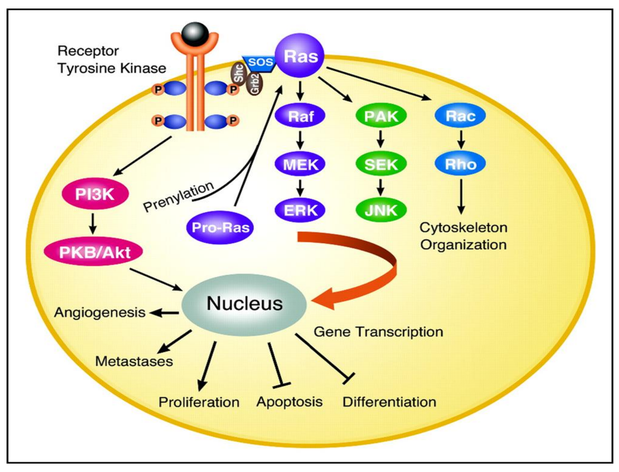

Figure 1. EGFR-KRAS- pathway

It is therefore essential to determine KRAS status prior to establishment of anti-EGFR therapy. Several recent studies have shown that KRAS mutation in CRC correlates with poor prognosis and high mitotic activity. Nevertheless, its relation to other histopathological and molecular parameters has not been well established.

Our first approach was to determine correlations between the KRAS status and 27 histopathological or molecular features in an univariate analysis using a retrospective series of 337 paraffin embedded CRC samples (Table 1).

METHODS

Patients. A cohort of 337 patients with primary colorectal adenocarcinoma was used for this retrospective study. Demographic data on this cohort may be summarized as follows: 200 patients (87%) were males and 137 (13%) were females; median age was 68.7±11.9 years (range 31-98).

Tissues. Specimens were routinely fixed in 10% buffered formalin and embedded in paraffin. Representative hematoxylin and eosin-stained (H&E) sections of each case were examined by two pathologists (average 4 slides per each tumor). Histopathological features were evaluated according to previously reported criteria. Ten 5µm thick sections of formalin-fixed, paraffin-embedded, tissues were used for each paired case (normal and tumoral samples) to perform manual scrapping. DNA was isolated using a proteinase K-phenol/chloroform protocol.

PCR and Sequencing Studies. PCR products were obtained from single reaction for KRAS (exon 1), BRAF and P53 entire exons 4 to 8. Mutational analysis was carried out by direct sequencing using the ABI PRISM® BigDye Terminator v1.1 Cycle Sequencing Kit (Figure 2).

Statistical Analyses. KRAS mutations were investigated for their association with clinicopathological features by Fisher's exact test on categorical variables, and by Kruskal-Wallis test on numerical variables. p-values were considered statistically significant when less than 0.05.

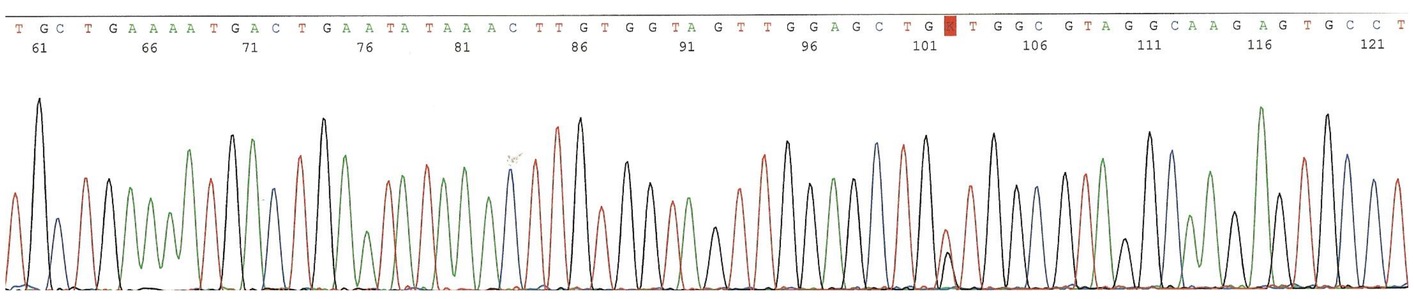

Figure 2. Example of KRAS mutation at codon 12

RESULTS

KRAS mutations were identified in 144 out of 337 (42.7%) CRC samples. BRAF V600E was found in 32 cases out of 318 studied (10.1%). In the subset of cases with KRAS mutations only one (0.7%) presented BRAF V600E, while 31 (16.9%) were found in the KRAS wild-type group (p<0.001). Out of the 144 cases with KRAS mutations, 72 cases (50%) contained P53 mutations. In the KRAS wild-type group, 133 cases (70%) harbour P53 mutations (p<0.001). The immunoexpression of P53 was also significantly higher in the KRAS wild-type group (p= 0.009). Significant association was also observed between the presence of KRAS mutations and a higher percentage of mucinous component (p=0.001). Similarly the presence of KRAS mutations was significantly associated with a lower percentage of solid component (p=0.010). No other significant correlations were found between the presence of KRAS mutations and the other histopathological features evaluated (Table 1).

CONCLUSIONS

KRAS (42.7%) and BRAF V600E (10.1%) mutations were found to be mutually exclusive (p< 0.001) according to published data. A significantly higher number of P53 mutated cases was found in the KRAS wild-type group, while there were equal numbers of P53 mutated and wild-type cases in the KRAS mutated group. This supports the hypothesis of P53 inactivation and KRAS mutations arising through independent pathways. The lack of association between KRAS and high grade parameters such as pT, tumour size and presence of lymph node metastasis, sustains the fact that KRAS mutations are an early event in colorectal carcinogenesis. The importance of the associations observed between the presence of KRAS mutations and the percentage of mucinous component and solid tumoral growth pattern must be further studied. A multivariate analysis would be required to further analyse all these correlations.

Categorical variable	KRAS WT (%) n=193	KRAS MUTAT (%) n=144	*p*-valor Fisher
Gender			0.911
Male	114 (33.8)	86 (25.6)	
Female	79 (23.4)	58 (17.2)	
Location			0.188
Proximal	57 (17)	55 (16.3)	
Distal	111 (32.9)	69 (20.5)	
Rectum	25 (7.4)	20 (5.9)	
Configuration			0.223
Exophytic	78 (23.1)	70 (20.8)	
Ulcerated	75 (22.3)	53 (15.7)	
Stenosing	40 (11.9)	21 (6.2)	
Extent of invasion (pT)			0.664
pT0	1 (0.3)	2 (0.6)	
pT1	15 (4.5)	8 (2.4)	
pT2	24 (7.1)	22 (6.6)	
pT3	92 (27.3)	62 (18.4)	
pT4	61 (18.1)	50 (14.8)	
Intramural TWVI			0.156
Present	70 (20.8)	41 (12.2)	
Absent	123 (36.5)	103 (30.6)	
Extramural TWVI			0.816
Present	63 (18.7)	49 (14.5)	
Absent	130 (38.6)	95 (28.2)	
Intramural VVI			0.396
Present	16 (4.7)	8 (2.4)	
Absent	177 (52.5)	136 (40.4)	
Extramural VVI			0.792
Present	45 (13.4)	31 (9.2)	
Absent	148 (43.9)	113 (33.5)	
Intramural PNI			0.738
Present	6 (1.8)	3 (0.9)	
Absent	187 (55.5)	141 (41.8)	
Extramural PNI			1.000
Present	25 (7.4)	18 (5.3)	
Absent	168 (49.9)	126 (37.4)	
Growth pattern			0.125
Expansive	68 (20.2)	39 (11.6)	
Infiltrative	125 (37.1)	105 (31.1)	
Crohn-like lymphoid reactivity			0.906
Present	61 (18.1)	47 (13.9)	
Absent	132 (39.2)	97 (28.8)	
TIL			1.000
Present	26 (7.7)	20 (5.9)	
Absent	167 (49.6)	124 (36.8)	
Residual adenoma			0.108
Present	61 (18.1)	58 (17.2)	
Absent	132 (39.2)	86 (25.5)	
BRAF V600E mutation			<0.001
Present	31 (9.7)	1 (0.3)	
Absent	152 (47.8)	134 (42.1)	
P53 mutation			0.003
Present	133 (39.8)	72 (21.6)	
Absent	57 (17.1)	72 (21.6)	
Numerical variable	Mean SD	Mean SD	*p-valor* Kruskal-Wallis
Age	68.4 12.3	69.2 11.3	0.492
Tumor size (mm)	40.4 18.1	42.4 21.6	0.498
Solid carcinoma (%)	12.1 22.6	6.3 13.4	0.010
Extracelular Mucinous component (%)	11.5 24.7	13.4 24.7	0.001
Intracelular Mucinous component (%)	0.4 3.7	0.1 0.7	0.845
Cribriform pattern (%)	7.3 14.9	9.2 18.0	0.834
Micropapillary pattern (%)	2.2 8.6	2.5 7.9	0.285
Microglandular pattern (%)	2.9 11.0	2.2 5.3	0.256
Nodal involvement (n)	2.4 4.4	1.8 3.2	0.375
Ki67 proliferative index (%)	59.7 21.3	57.0 21.7	0.310
p53 overexpression (%)	41.9 36.2	29.5 34.1	0.009

Table 1. Correlation between KRAS status and clinicopathological features

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