CORRELATION BETWEEN KRAS MUTATIONS AND CLINICOPATHOLOGICAL FEATURES IN COLORECTAL CARCINOMAS. A MULTIVARIATE ANALYSIS.

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BACKGROUND

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. 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 clinicopathological and molecular parameters has not been well established.

The aim of this study was to further analyse with a multivariate analysis the correlations observed in our initial univariate approach between KRAS mutational status and clinicopathological parameters.The 29 clinicopathological and molecular features studied are summarized on Table 1.

METHODS

Patients. A cohort of 308 patients with primary colorectal adenocarcinoma was used for this retrospective study.

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 were used for each paired case (normal and tumor samples) to perform manual scrapping. DNA was isolated using a proteinase K-phenol/chloroform protocol.

Microsatellite Instability Analysis. MSI status was evaluated using a panel of 11 microsatellites: BAT25, BAT26, D5S346, D2S123 and D17S250; five additional microsatellites originally aimed at detecting LOH status of chromosome 18q and a microsatellite at the TP53 locus on 17p (P53CA). Fluorescent amplicons were analyzed on an automated ABI PRISM® 310 Genetic Analyzer using the GeneScan software.

PCR and Sequencing Studies. PCR products were obtained from single reaction for KRAS (exon 1), BRAF (exon 15) and P53 exons 4 to 8. Mutational analysis was carried out by direct sequencing.

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. For each of the 10 validation sets, where it is successively excluded 10% of full data set, the *glmnet* package with Schwartz criteria was employed.

RESULTS

The *glmnet* cross validation analysis carried out with the global series, involving a total of 1000 random data sets, found that V600E BRAF mutation, overexpression of p53 and mutational status of p53 were strongly associated with KRAS status being present respectively in 1000, 727 and 1000 of the generated models (Highlighted on Table 1).

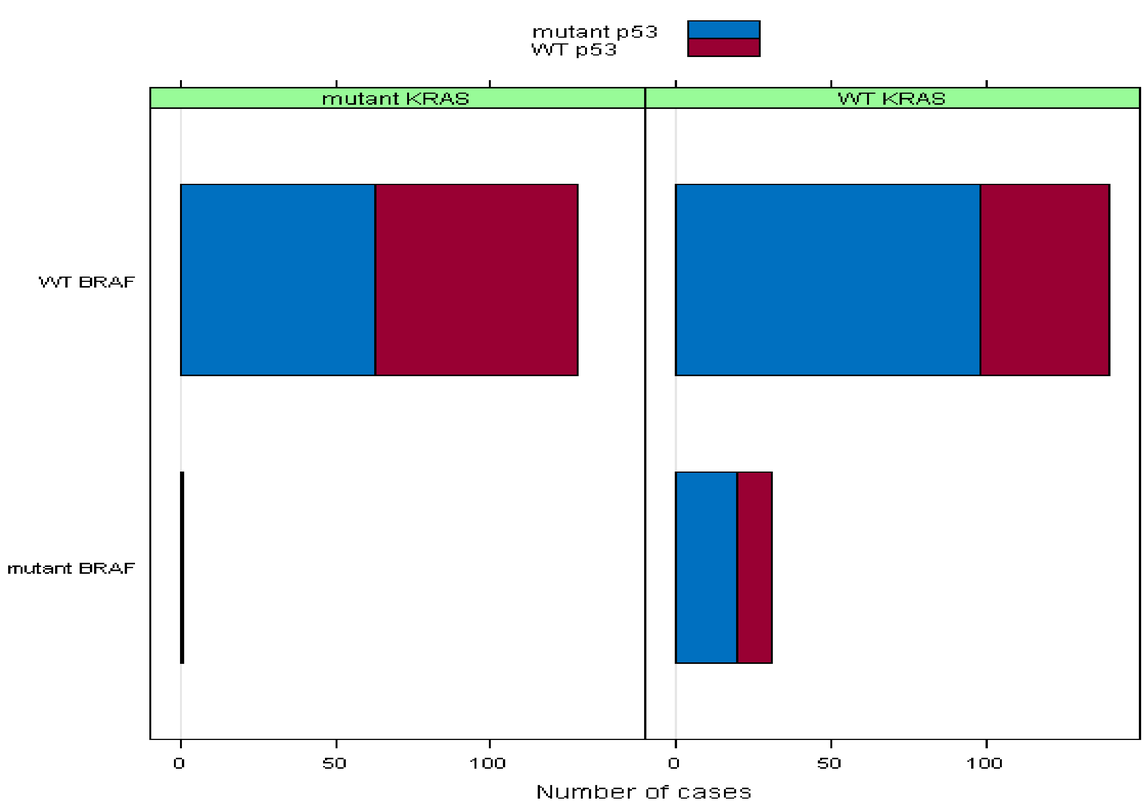

Figure 1. Association between KRAS mutational status and BRAF and p53 mutations

Categorical variable	WT KRAS (%) n=178 (57.8)	MUTANT KRAS (%) n=130 (42.2)
Gender		
Male	106 (34.4)	74 (24.0)
Female	72 (23.4)	56 (18.2)
Location		
Proximal	55 (17.9)	52 (16.9)
Distal	63 (20.4)	39 (12.7)
Rectum	53 (17.2)	38 (12.3)
Unknown	7 (2.3)	1 (0.3)
Configuration		
Exophytic	55 (17.9)	54 (17.5)
Ulcerated	69 (22.4)	46 (15.0)
Stenosing	37 (12.0)	17 (5.5)
Polipoid	17 (5.5)	13 (4.2)
Extent of invasion (pT)		
pT1	13 (4.2)	8 (2.6)
pT2	22 (7.2)	21 (6.8)
pT3ab	57 (18.5)	37 (12.0)
pT3cd	30 (9.8)	19 (6.2)
pT4a	2 (0.6)	4 (1.3)
pT4b	54 (17.5)	41 (13.3)
Intramural TWVI		
Present	66 (21.4)	37 (12.0)
Absent	112 (36.5)	93 (30.2)
Extramural TWVI		
Present	60 (19.5)	44 (14.3)
Absent	118 (38.3)	86 (28.0)
Intramural VVI		
Present	14 (4.5)	7 (2.3)
Absent	164 (53.2)	123 (40.0)
Extramural VVI		
Present	42 (13.6)	27 (8.8)
Absent	136 (44.2)	103 (33.4)
Intramural PNI		
Present	5 (1.6)	2 (0.6)
Absent	173 (56.2)	128 (41.6)
Extramural PNI		
Present	24 (7.8)	13 (4.2)
Absent	154 (50.0)	117 (38.0)
Growth pattern		
Expansive	62 (20.1)	36 (11.7)
Infiltrative	116 (37.7)	94 (30.5)
Crohn-like lymphoid reactivity		
Present	56 (18.2)	44 (14.3)
Absent	122 (39.6)	86 (28.0)
TIL		
Present	24 (7.8)	19 (6.2)
Absent	154 (50.0)	111(36.0)
Residual adenoma		
Present	53 (17.2)	54 (17.5)
Absent	125 (40.6)	76 (24.7)
BRAF V600E mutation		
Present	31 (10.1)	1 (0.3)
Absent	147 (47.7)	129 (41.9)
p53 mutation		
Present	124 (40.3)	63 (20.5)
Absent	54 (17.5)	67 (21.7)
MSI		
MSI-H	25 (8.1)	7 (2.3)
MSI-L	24 (7.8)	19 (6.2)
MSS	129 (41.9)	104 (33.8)
MMR (IHC)		
Conserved expression	158 (51.3)	125 (40.6)
Loss of expression	20 (6.5)	5 (1.6)

Numerical variable	Mean	SD	Mean	SD
Age	68.2	12.4	69.8	11.1
Tumor size (mm)	40.9	17.6	43.5	21.9
Solid carcinoma (%)	12.9	23.3	6.6	14.0
Extracelular mucinous component (%)	11.6	24.7	14.6	25.7
Intracelular mucinous component (%)	0.5	3.8	0.1	0.7
Cribriform pattern (%)	7.5	15.3	9.3	18.6
Micropapillary pattern (%)	2.2	8.9	2.4	8.2
Microglandular pattern (%)	3.1	11.5	2.0	5.1
Nodal involvement (n)	2.5	4.4	1.9	3.2
Ki67 proliferative index (%)	59.7	21.3	56.5	21.0
p53 overexpression (%)	42.1	35.9	28.2	33.5

Table 1. Correlation between KRAS status and clinicopathological features

CONCLUSIONS

- This multivariant approach confirmed the results of our previous univariant study. KRAS wild-type status is associated with overexpression of p53 as well as presence of BRAF and p53 mutations (Figure 1).
- This supports the hypothesis of p53 inactivation and KRAS mutations arising through independent pathways.
- Our multivariate analysis did not allow us to confirm the association observed in our initial univariate analysis between KRAS mutational status and presence of solid and extracelullar mucinous components.
- The lack of association between KRAS and parameters such as pT, tumour size and presence of lymph node metastasis, is in agreement with the fact that KRAS mutations are an early event in colorectal carcinogenesis.

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