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Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study

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Summary Researchers worldwide with information about the Kirsten ras (Ki-ras) tumour genotype and outcome of patients with colorectal cancer were invited to provide that data in a schematized format for inclusion in a collaborative database called RASCAL (The Kirsten ras in-colorectal-cancer collaborative group). Our results from 2721 such patients have been presented previously and for the first time in any common cancer, showed conclusively that different gene mutations have different impacts on outcome, even when the mutations occur at the same site on the genome. To explore the effect of Ki-ras mutations at different stages of colorectal cancer, more patients were recruited to the database, which was reanalysed when information on 4268 patients from 42 centres in 21 countries had been entered. After predetermined exclusion criteria were applied, data on 3439 patients were entered into a multivariate analysis. This found that of the 12 possible mutations on codons 12 and 13 of Kirsten ras, only one mutation on codon 12, glycine to valine, found in 8.6% of all patients, had a statistically significant impact on failure-free survival ($P = 0.004$, HR 1.3) and overall survival ($P = 0.008$, HR 1.29). This mutation appeared to have a greater impact on outcome in Dukes' C cancers (failure-free survival, $P = 0.008$, HR 1.5; overall survival $P = 0.02$, HR 1.45) than in Dukes' B tumours (failure-free survival, $P = 0.46$, HR 1.12; overall survival $P = 0.36$, HR 1.15). Ki-ras mutations may occur early in the development of pre-cancerous adenomas in the colon and rectum. However, this collaborative study suggests that not only is the presence of a codon 12 glycine to valine mutation important for cancer progression but also that it may predispose to more aggressive biological behaviour in patients with advanced colorectal cancer.

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It is widely accepted that mutations in the Kirsten ras (Ki-ras) gene in patients with colorectal cancer develop early in the progression from adenoma to carcinoma. Our first collaborative study including 2721 patients, clarified that Ki-ras mutations are not only

important for the development of colorectal cancer but also for its progression (Andreyev et al, 1998). We showed that the presence of a mutation in Ki-ras increased risk of death by 26% ($P = 0.004$). Secondly, for the first time in any common cancer, we conclusively showed that different mutations may have different effects. For example, any mutation of guanine (G) to thymidine (T) but not to adenine (A) or to cytosine (C) increased the risk of death by 44% ($P = 0.0002$). When individual mutations were evaluated, one single mutation found in just under 10% of all patients with colorectal cancer – that of glycine to valine on codon 12 – was an independent risk factor for recurrence ($P = 0.0008$) and death ($P = 0.0019$). Thus, Ki-ras mutations were associated with an increased risk of relapse and death, and some mutations were found to be more aggressive than others.

The size of our collaborative database also allowed definitive conclusions to be drawn on a number of other unresolved issues. In particular, we found that mutations were not associated with gender, age, tumour site or Dukes' stage and that mutation rates seen in patients with sporadic tumours were comparable to the rates observed in patients with a predisposing cause for their cancer.

One intriguing possibility arising from our first study was that the aggressive mutations were playing a different role in early tumours compared to more advanced tumours. However, the first RASCAL (The Kirsten ras in-colorectal-cancer collaborative group) study was too small to define this point and it became obvious that additional patients would need to be recruited to the database so that this second study could explore further the role of the Ki-ras mutation at different stages of colorectal cancer.

METHODS

Patients

At least 2 invitations were sent to all researchers who had published original data in English or were known to have unpublished data on the significance of the Ki-ras gene in patients with colorectal adenocarcinoma. They were invited to participate in a collaborative register collecting original clinical data from such patients.

Participants were required to complete a questionnaire for each patient and details were entered into a database. All collaborators were asked to ensure that information in 3 areas in particular was as complete as possible. The following information was requested: (1) the genotype of the Ki-ras gene in the primary tumour at codons 12 and 13, (2) the date, Dukes' stage and apparent immediate outcome of any surgery for that cancer and (3) dates of follow-up and long-term outcome. Specific causes of death and dates of recurrence, if relevant, were also sought. All data were coded so that patient identity was only known to their physicians and were entered by one statistician (ARN) into a database called RASCAL.

Statistics

Survival curves were generated using the product-limit method of Kaplan–Meier. The log rank test was used to evaluate differences in failure-free survival and overall survival curves. Failure-free survival was defined as the time from operation to relapse or death from any cause apart from peri-operative deaths. Overall survival was defined as the time from operation to death from any cause. Chi-square tests were used for comparison of categorical data. In view of the multiple statistical analyses performed and the large number of patients, only values where $P < 0.01$ were considered significant. Multivariate analysis was performed using Cox's model for proportional hazards survival analysis. Hazard ratios (HRs) and 95% confidence intervals (CIs) of the hazard ratios were calculated from the individual Cox multivariate analyses. All P-values were calculated from the improvement in log likelihood and were expressed as two-sided.

Bias introduced by the findings of different centres was examined using the test for heterogeneity across centres. The difference in prevalence of mutations between centres was examined using a chi-square test. Centre was treated as a random effect and age treated as a continuous variable in the model. Dukes' stage was analysed using dummy variables for each stage relative to Dukes' A. A separate model was generated for each of the mutation types. This allowed a hazard ratio to be calculated for each type separately, after controlling for centre, age and Dukes' stage. The model was repeated for the Dukes' B and then Duke's C patients separately to get an estimate of the effect on the individual stages.

RESULTS

Patient selection

Data on 4268 patients from 42 different centres in 21 countries were entered onto the RASCAL database (Table 1). Our earlier study had previously reported on 2721 of these in whom there had been clinical outcome data in 2445.

Patients excluded from further analysis were those with missing age ($n = 203$) or Dukes' stage ($n = 75$). Perioperative deaths ($n = 76$) are all included in the database but the deaths are censored and do not count as events. Data from one centre ($n = 34$) were removed as patient autopsy specimens were used. Centres that did not provide information about the exact mutation type were also excluded ($n = 488$). Where a centre had a minority of missing mutation types, only the missing mutation patients were removed. Patients were also excluded if information provided for codon 12 included only mutations and data on codon 13 was missing ($n = 49$ from 9 centres excluding 1.3–34.3% of their data). After these exclusions, the number of patients used for the analysis

Table 1 Characteristics of 4268 patients from 42 different centres in 21 countries enrolled in the RASCAL database

Characteristic	Male	Female	Unknown	Total
No. of patients	2263	1977	28	4268
Median age in years (range)	68 (17–95)	68 (19–103)	–	–
Dukes' stage				
A	334	304	5	643
B	941	819	9	1769
C	659	589	8	1256
D	287	238	0	525
Unknown	42	27	6	75
Alive at last follow up	1228	1137	26	2391
Cause of death				
Peri-operative	48	28	0	76
Cancer	600	559	1	1160
Unrelated to cancer	191	116	0	307
Unknown	196	137	1	334