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Tumorigenesis

RAF/*RAS* oncogenes and mismatch-repair status

Genes of the *RAF* family encode kinases that are regulated by Ras and mediate cellular responses to growth signals. Activating mutations in one *RAF* gene, *BRAF*, have been found in a high proportion of melanomas and in a small fraction of other cancers[1]. Here we show that *BRAF* mutations in colorectal cancers occur only in tumours that do not carry mutations in a *RAS* gene known as *KRAS*, and that *BRAF* mutation is linked to the proficiency of these tumours in repairing mismatched bases in DNA. Our results not only provide genetic support for the idea that mutations in *BRAF* and *KRAS* exert equivalent effects in tumorigenesis[2], but also emphasize the role of repair processes in establishing the mutation spectra that underpin human cancer.

To determine how alterations in *BRAF* and *KRAS* might affect one another, we systematically evaluated mutations in these genes in 330 colorectal tumours (Table 1). We identified 32 mutations in *BRAF*: 28 cases with thymine-to-adenine (T–A) transversions at nucleotide position 1,796 (corresponding to an amino-acid swap of glutamate for valine at residue 599; V599E), and one case each of a guanine-to-thymine (G–T) transversion at nucleotide 1,382 (R461I), a T–G transversion at nucleotide 1,385 (I462S), a G–A transition at nucleotide 1,388 (G463E), and an A–G transition at nucleotide 1,798 (K600E). All but two of these mutations seemed to be heterozygous, and in all 20 cases for which normal tissue was available, the mutations were shown to be somatic. In the same set of tumours, there were 169 mutations in *KRAS*, including alterations to codons 12, 13, 59 and 61. No tumour exhibited mutations in both *BRAF* and *KRAS*.

Mutations in either *BRAF* or *KRAS* occurred in all Duke's stages of cancer (results not shown) and also in premalignant lesions. Mutations in both genes seemed to be more common in adenomas larger than 1 cm across than they were in smaller adenomas.

There was also a striking difference in the frequency of *BRAF* mutations between cancers with and without mismatch-repair (MMR) deficiency ($P < 10^{-6}$, χ^2 test; Table 1). All but one of the 15 *BRAF* mutations identified in MMR-deficient cases resulted in a V599E substitution.

These results provide strong support for the hypothesis that *BRAF* and *KRAS* mutations are equivalent in their tumorigenic effects[2]. Both genes seem to be mutated at a similar phase of tumorigenesis, after initiation but before malignant conversion. Moreover, we found no tumour that concurrently contained both *BRAF* and *KRAS* mutations. In view of the large number of mutations of both genes found in colorectal cancers, this observation is highly statistically significant ($P < 10^{-6}$, χ^2-test) and cannot be easily explained in other ways. This conclusion could not have been reached through the study of melanomas or of most other tumour types in which only one of the two genes is commonly mutated. It is consistent with biochemical observations[3] and was suggested by Davies *et al.*[1].

Our results also show that MMR-deficient tumours have a very high incidence of *BRAF* mutations and a lower incidence of *KRAS* mutations compared with MMR-proficient colorectal cancers. This is consistent with the idea that both tumour types progress through the same biochemical pathways, but that the mutation spectrum depends on the nature of the underlying genetic instability[4]. The V599E mutation is the most frequent nucleotide substitution ever identified in a repair-deficient tumour.

The only other tumour type with a *BRAF*-mutation frequency as high as that seen in MMR-deficient colorectal cancers is melanoma[1]. Melanomas and MMR-deficient colorectal cancers also share a high incidence of mutations in the oncogene that encodes β-catenin[5,6]. It will be interesting to see whether melanomas have a repair defect that makes them susceptible to the types of mutation found in MMR-deficient colorectal cancers, and to determine what structural or sequence elements surrounding *BRAF* codon 599 make it prone to mutagenesis in a repair-deficient background.

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Table 1 *BRAF* and *KRAS* mutations in colorectal tumours

Tumours	*No. of cases*	BRAF *mutation*	KRAS *mutation*
All types	330	32 (10%)	169 (51%)
BRAF mutants	1	R461I	WT
	1	I462S	WT
	1	G463E	WT
	28	V599E	WT
	1	K600E	WT
KRAS mutants	169	WT	MUT
Other	129	WT	WT
Clinical cancers	276	30 (11%)	154 (56%)
Adenomas > 1 cm	20	2 (10%)	12 (60%)
Adenomas ⩽ 1 cm	34	0 (0%)	3 (9%)
MMR-deficient cancers	49	15 (31%)	21 (43%)
MMR-proficient cancers	227	15 (7%)	133 (59%)

DNA was purified from microdissected primary tumours ($n = 54$), first-passage xenografts ($n = 189$) or cell lines ($n = 87$) as described[7]. The complete coding sequences of exons 11 and 15 of *BRAF* and exons 2 and 3 of *KRAS* were amplified by polymerase chain reaction using intronic primers and the products were sequenced as described[8]. Mutations were identified using the Mutation Explorer package (SoftGenetics). This strategy allowed us to identify all mutations previously known to occur in these two genes. Mismatch-repair (MMR) deficiency was assessed by analysis of microsatellite instability, using the *BAT26* marker and at least 12 microsatellite repeat markers[9]. WT, wild-type sequence; MUT, mutations in codons 12, 13, 59 or 61 in *KRAS*.