Analysis of Ha-ras 1 allele frequencies in hereditary non-polyposis colorectal cancer

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Abstract
Restriction enzyme digests of genomic DNA show multiple alleles of common, intermediate, and rare frequencies at the minisatellite locus of Ha-ras. It has been suggested that a higher frequency of rare alleles is associated with the presence of colorectal and other types of cancer. This study investigated the distribution of Ha-ras alleles in 40 members of hereditary non-polyposis colorectal carcinoma (HNPCC) families and in 34 cancer-free subjects (spouses). There was no difference in rare allele frequency between the cancer group and cancer-free group ($\chi^2 = 0.25$, not significant).

Methods
Peripheral blood samples were collected from 40 white cancer patients who were members of 15 different HNPCC families as defined by the Amsterdam criteria. Neoplasms detected in these patients included colon cancers (32), adenomas (5), and cancers of the endometrium (6), ovary (6), breast (2), cervix (1), brain (1), small bowel (1), and skin (3). Twelve patients had multiple cancers. The control group consisted of 34 unaffected white subjects who were spouses of HNPCC family members. All subjects gave written informed consent in accordance with the guidelines of the Auckland Hospital ethics committee.

High molecular weight DNA was prepared from peripheral blood samples by standard methods of sodium dodecylsulphate-proteinase K digestion followed by phenol chloroform extraction and ethanol precipitation. Three to 5 µg of DNA was digested with 20–30 units of either MspI or BamHI under conditions recommended by the manufacturer. DNA fragments were separated by electrophoresis on 1-2% gels for the MspI digests and 0-7% gels for the BamHI digests and transferred to Gene Screen Plus nylon membranes after denaturation and neutralisation. Filters were hybridised to a 6-4 kb genomic c-Ha-ras 1 probe obtained from the American Type Culture Collection.

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Probes were labelled with $^{32}$P by random primer extension, and hybridisation conditions were as previously described. Autoradiography was performed at $-70\degree C$ for one to three days using intensifying screens. Allele sizes were calculated from ethidium-bromide stained EcoRI digested bacteriophage SPP1 DNA, and $^{32}$P-labelled 1 kb DNA ladder. Control samples consisting of alleles $a_1$, $a_2$, and $a_3$ were run as standards on all gels.

**Results**

After digestion with MspI, the four common progenitor alleles $a_1$, $a_2$, $a_3$, and $a_4$, were found in similar frequencies in both the cancer and control groups. Differences as small as 20 bp could be distinguished with MspI digests, and these were used to identify the rare alleles. BamHI digests did not permit accurate size determinations and were used only to confirm homozygosity or heterozygosity of MspI alleles. An additional 12 alleles of intermediate and rare frequencies were identified.

The data for the cancer group were collected from members of 15 different HNPCC families, with one to 12 patients from each family. As related members are likely to share alleles, allele frequencies within families are not independent. To obtain a set of independent findings we identified a subset of the cancer group consisting of the proband from each family. Table I summarises these results. Alleles were named according to the nomenclature of Krontiris et al. The frequency of rare alleles did not seem to be higher in the cancer proband group than in the control group ($\chi^2=0.25$, not significant).

Data from all the cancer patients were also analysed by taking allele frequencies within each family and weighting them by the inverse of family size, so that their contribution summed to one. This gave an effective total of 30 alleles and 15 genotypes, with no difference in the allele frequencies between the control group and cancer group ($\chi^2=0.26$, not significant).

In the analysis of genotypes, shown in Table II, a distinction was made between subjects having two common alleles, and those having one or two intermediate or rare alleles. The only notable difference between the cancer and control groups was the $a_1/a_4$ frequency, which was one of 34 (2.9%) in the control group and eight of 40 (20%) in the cancer group. Six of eight $a_1/a_4$ genotypes in the cancer group were members of the same pedigree while six other members of this family had different genotypes. There was no difference in the frequencies of common or rare genotypes between the control group and the cancer proband group ($\chi^2=0.76$, not significant).

**Discussion**

Studies on Ha-ras alleles in colorectal cancer have produced contradictory results. Klingel et al found an increased frequency of rare alleles in a study population of unrelated colorectal carcinoma patients. Our results are more in agreement with those of Wyllie et al who found no significant difference in rare allele frequency between their cancer and control groups, although it was not specifically stated whether the cancer group consisted of sporadic or familial colorectal cancers.

It is of interest that Wyllie et al found the combined frequency of alleles $a_3$ and $a_4$ to be twice as high in the cancer group. In our study the $a_1/a_4$ genotype is present in 20% of the cancer group, but in only 2-9% of the control group. Six of eight $a_1/a_4$ genotypes are from family 3, however, the largest family used in this study, while the two remaining $a_1/a_4$ genotypes were from two different families. The high frequency of this genotype could simply result from the large contribution from a single family, particularly in view of the comparatively small numbers in this study.

The case control study and meta-analysis of Krontiris et al suggest that Ha-ras rare alleles are indicative of an increased risk of cancer. Previous studies have all concerned unrelated cancer patients. This study differs in that it looked at rare allele frequencies in familial cancer. Clinical features including age at onset of cancer and multiple cancers were not associated with Ha-ras allele status. Thus it is possible that the mechanisms of pathogenesis are different in sporadic and familial cancer, and rare Ha-ras alleles may not play a part in the pathogenesis of HNPCC.

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