Stability of critical genetic lesions in human colorectal carcinoma xenografts

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Several genetic lesions are associated with the genesis of human colorectal carcinomas including mutational activation of Ki-ras and p53, and loss of heterozygosity involving presumptive oncosuppressor loci on 5q21, 17p13, and 18q22 (Fearon & Vogelstein, 1990). Abnormalities at the 17p13 locus are recorded in many types of tumours (Nigro et al., 1989) but there is no evidence at present that the 5q and 18q loci are involved in sporadic neoplasia other than that of colorectal mucosa. Although the 17p13 locus is probably congenic with p53, and the product of the 18q22 gene appears to be a cell adhesion molecule, the function for all of these genes in oncosuppression is poorly understood. The ideal vehicle for assay and analysis of these unknown gene functions would be a colorectal cancer cell line in which the oncosuppressor genes were known to be aberrant or inactive. In theory, replacement of even one copy of the appropriate intact oncosuppressor gene might then restore phenotype to normality. Recent studies have demonstrated oncosuppression of this type on introduction of normal DNA from the retinoblastoma and Wilms’ susceptibility loci, into tumour cell lines (Huang et al., 1988; Weissman et al., 1987). However, for none of the long-established colorectal cancer lines readily available is status at critical oncosuppressor loci known. Moreover long-established lines commonly acquire additional genetic rearrangements during culture (Brattain et al., 1985; Park et al., 1987), which may not be reversible by correction of the original oncosuppressor defect alone. We therefore set out to develop a series of lines from primary colorectal tumours, defined both in terms of status at oncosuppressor loci on chromosomes 5, 17 and 18, and the stability of each locus on serial passage.

Portions of carcinoma obtained from surgical resection specimens were xenografted as described by Lewko et al. (1989), into pairs of immunosuppressed (thymectomised, irradiated and arabinoside-C treated) CBA mice (Steel et al., 1978), with the modification that portions no greater than 5 mm³ were implanted dorsally at initial xenograft and at subsequent transplantations.

From a consecutive series of 28 cancers implanted, 11 grew to passage and eight were xenografted for more than five passages, one of which has now reached passage 16. At each serial passage excess tumour tissue was divided for analysis allowing the characterisation of xenografted tumours at various passage intervals, and the comparison of these with the primary tumour (portions of which were always stored both frozen and fixed at original resection). Haematoxylin and eosin stained paraffin sections of both primary tumour and xenografts fixed in 10% buffered formalin at all passages, showed that the original histological pattern for each primary tumour was conserved throughout serial passage. Although most of the lines fell into the class of adenocarcinomas of average degree of differentiation, extremes of both good and very poor differentiation were represented (Figure 1). No metastases were observed in mice.

To study the status of alleles closely linked to each of three putative colon-associated oncosuppressor loci, genomic DNA was prepared from frozen xenografted tumours at various passages, and compared with DNA from primary tumour tissue and normal colorectal mucosa or peripheral blood leukocytes from the donor patient. Methods for DNA preparation, Southern blotting and probes and conditions for hybridisation have been previously published (Ashton-

![Figure 1](https://example.com/figure1.png)  
**Figure 1** Histological comparisons of original colorectal tumours with the corresponding xenografts. 

| a | b | c | d | e | f |
|---|---|---|---|---|---|
| A poorly differentiated adenocarcinoma AGDU. | Corresponding xenograft at 5th passage XAGDU4. | A well differentiated adenocarcinoma MUCO. | Corresponding xenograft at 11th passage XMUCO10. | A moderately differentiated adenocarcinoma CHKE. | Corresponding xenograft at 6th passage XCHKE5. |

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passage

Table I summarises allelic losses or retentions found for the parental normal and tumour DNA pairs at each locus. In all cases studied, the allelic status of xenograft DNA was unchanged from that of its primary tumour. For eight tumours this genetic stability was demonstrated over at least four xenograft passages (approximately 4 months) and in two (one of which showed allelic retention at 5q and 17p) over 11 passages (approximately 11 months). A variety of combinations of allelic losses and retentions are represented, broadly similar to those observed by ourselves and others in large unselected series of primary tumours (Ashton-Rickardt et al., 1989; Vogelstein et al., 1988). It appears from these observations that the selection pressures which encourage growth of human colorectal cancer xenografts in immunosuppressed animals do not include allelic losses around the oncosuppressor loci commonly affected in human carcinogenesis in situ.

Although our primary aim was to establish the stability of the lines at critical oncosuppressor loci, DNA ploidy was measured by flow cytometry as a global, if crude, index of total nuclear DNA content. Frozen tissues from both passage 5 xenografts and primary tumour were dissociated and stained with 0.62 M propidium iodide as described by Vindelov (1983), and analysed at an excitation wavelength of 488 nm in a Coulter Epics CS flow cytometer. On the basis of an internal chicken red blood cell standard, the presence of a human diploid peak could be confirmed in every case. This was given a ploidy value of 1, and any additional peaks were then assumed to be aneuploid, and their DNA index (DI) derived from their position relative to the diploid peak. In all cases, xenograft DI was similar to that of the relevant primary tumour (example shown in Figure 2), including four tumours remaining diploid after five or nine serial passages in mice, and one after 14 passages (15 months). A similarly high degree of conservation of genetic stability throughout xenograft passage in immunodeficient mice has been confirmed both karyotypically and at the genetic level by Lefrancois et al. (1989).

The demonstrated lack of genetic change for defined oncosuppressor loci qualifies these xenografts as favourable tumour models for analysis of oncosuppressor function, by re-introduction of the normal genes. Since growth in vitro is a prerequisite for genetic manipulation, tumour tissues were removed immediately on to ice from animals at or following passage 5 were processed and seeded as primary cultures on collagen coated flasks with mouse feeder cells, as described by Paraseva (1984). Epithelial cells from every xenograft line grew in vitro as flat islands of cells spreading radially across the collagen surface towards neighbouring islands (Figure 3). Two slightly faster growing lines were observed over three passages in vitro, and a third has now grown rapidly over 100 days, requiring a weekly split ratio of up to 1:20. On subcutaneous re-injection into immunosuppressed mice, these cells continued to show the same allelic status and morphology as the primary tumour.

In conclusion, the xenograft lines described above represent a variety of defined and stable combinations of oncosuppressor gene status and will therefore provide a useful resource for the study of the functions of the genes involved in human colorectal cancer, and their interactions with each other.

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Table I: Allelic status at three oncosuppressor loci, and DNA index for a set of 11 xenografts.

| Line | 5q | 17p | 18q | DNA Index |
|------|----|-----|-----|-----------|
| GRBO* | R (5) | R (4) | R (2) | 1.0 (5) |
| MUCO* | R (10) | R (10) | 1.0 (14) |
| RHSP* | L (10) | L (10) | 1.7–1.9 (12) |
| CHKE* | R (4) | R (4) | R (3) | 1.1–1.2 (9) |
| DABU* | R (5) | R (5) | N/ | 1.0 (5) |
| ARNE* | R (4) | L (5) | L (5) | 1.5–1.6 (5) |
| MASM* | R (5) | R (5) | R (5) | 1.5–1.9 (9) |
| JOMcL* | R (4) | R (4) | N/ | 1.0 (5) |
| JACA | L (2) | L (2) | L (2) | 1.6 |
| CARO | L (1) | L (1) | L (1) | 1.4 |
| JOWR | R (1) | R (1) | R (1) | 1.0 |

* = highest xenograft passage no. analysed; N/ = not done; N/ = non-informative (patient homozygous for alleles studied); R = allele retained; L = allele lost; * = line xenografted over more than five passages. In all cases the allelic status of the xenografts throughout passage was identical with that of the primary tumour.
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