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Establishment and Characterization of an Epithelial Cell Line with Quasi-normal Chromosomes from a Tubular Adenoma of a Familial Polyposis Coli Patient

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An epithelial cell line designated FPCK-1 has been established from a tubular adenoma developing in a male familial polyposis coli (FPC) patient. The FPCK-1 cells grow very slowly with adundant mucus production and have been maintained stably for 3 years in culture. No growth was evident either in soft agar or nude mice. FPCK-1 cells present a normal male karyotype and do not show loss of specific loci on chromosomes 5, 17, 18, and 22 which have been reported to be lost frequently in human colon carcinomas. The cells have neither a point mutation on codon 12 of K-*ras* gene nor gene amplification of *myc*, c-H-*ras*, and/or c-K-*ras* genes. These results thus suggest the existence of hitherto unknown causative event(s) underlying adenoma development in FPC patients. The FPCK-1 cell line should prove useful for further analytical investigation of the multiple steps involved in human colon carcinogenesis.

Key words: Epithelial cell line — Colon carcinogenesis — Familial polyposis coli — Adenoma — Multistep carcinogenesis

Growing evidence at the molecular level for multistep progression of carcinogenesis has recently been accumulated. The developmental processes of human colorectal cancer, especially in familial polyposis coli (FPC)[8] patients have been the subject of several informative models aimed at the analysis of multistep carcinogenesis.[1-6] FPC is an autosomal dominant genetic disease and the numerous adenomatous polyps which develop at an early stage of the patient's life later frequently progress to malignant carcinomas.[7] It has been suggested that loss of the FPC gene on chromosome 5 may be the initial event in human colonic carcinogenesis followed by c-K-*ras* activation and loss of specific loci of chromosomes 17, 18, and others in succession to lead to overt malignancy.[1-4, 6-10] A deletion of chromosome 22 in colonic carcinomas has also been pointed out.[4, 10] However, there still remains much to be confirmed or clarified as to the actual significance and the order of occurrence of these genetic changes in colon carcinogenesis.

In vitro systems have been shown to be excellent models for analyzing multistep carcinogenesis.[11] However, despite many attempts, only a few epithelial cell lines have been established from FPC adenomas.[12, 13] Indeed, there is some doubt as to whether the reported cell line which has been used for studies of multistep progression is really representative of early phase adenoma cells; the cell line was characterized by rapid progression, considerable chromosomal aberrations and K-*ras* mutation that could happen in the late phase of colon carcinogenesis.[3, 6, 10] Therefore, establishment of stable adenoma cell lines with more normal traits is a basic requirement. The present paper communicates the establishment of a promising cell line from an FPC adenoma with quasi-normal genetic and phenotypic traits.

A tubular adenoma measuring 0.5×0.5 cm in size was removed from the colon of a 34-year-old male FPC patient in October, 1987. The histology of the original tissue was interpreted by our pathologists as indicating tubular adenoma with generally moderate atypia and only focal severe atypia (Fig. 1). Fresh tissue was finely minced and seeded in fifteen 60 mm plastic dishes supplied with a 6052 medium supplemented with 1% dialyzed fetal calf serum, 5 μg/ml insulin, 5 μg/ml transferrin, 5 ng/ml selenium, and 10 ng/ml epidermal growth factor. The composition of the 6052 medium was 20% MCDB152 medium[14] and 80% DM160 medium[15] without $CaCl_2$, this being supplemented with 80% of the mixture of amino acids and vitamins of the DM160 medium. The calcium ion concentration was adjusted to 0.3 m*M*.

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[8] Abbreviation: FPC, familial polyposis coli.

In 5 dishes epithelial outgrowth was observed in 2–3 weeks. The cells were collected and pooled for the first subculture after 2 months. The cell line, later designated FPCK-1, has been maintained at a splitting ratio of 1:2 and then 2:3 every 2 months using Dispase as a detaching enzyme to release cell sheets from culture dishes. To date FPCK-1 cells have remained in culture for 3 years and are in the 22nd passage at the time of writing. The cells consistently form similar epithelial growth patterns in sheets (Fig. 2) and Alcian blue-PAS staining reveals abundant production of mucus. No chromosomal abnormalities were detectable at the 6th passage (June, 1988) (Fig. 3) and the adenoma cells did not grow in soft agar at that time. At passage 14 (May, 1989) the cells

Fig. 1. Light microscopy of the FPC patient's adenoma tissue that was transferred to culture (hematoxylin and eosin staining). For diagnosis, see the text. ×70.

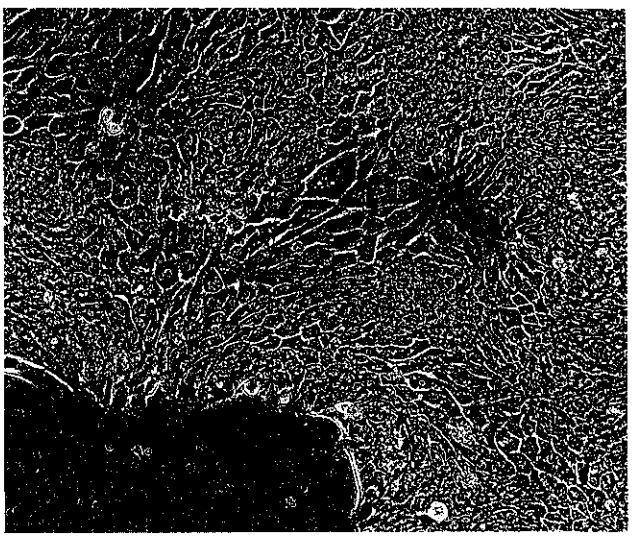

Fig. 2. A representative phase-contrast microscopic picture of FPCK-1. ×110.

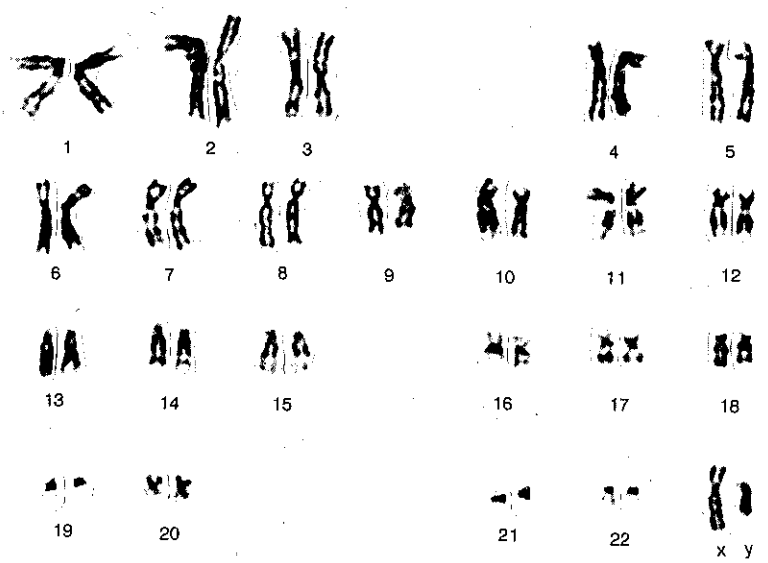

Fig. 3. Representative trypsin-produced G-banding karyotype of FPCK-1 at passage 6. Eight metaphase spreads were subjected to karyotypic analysis and all appear to be normal. There were no marker chromosomes or chromosomal aberrations.

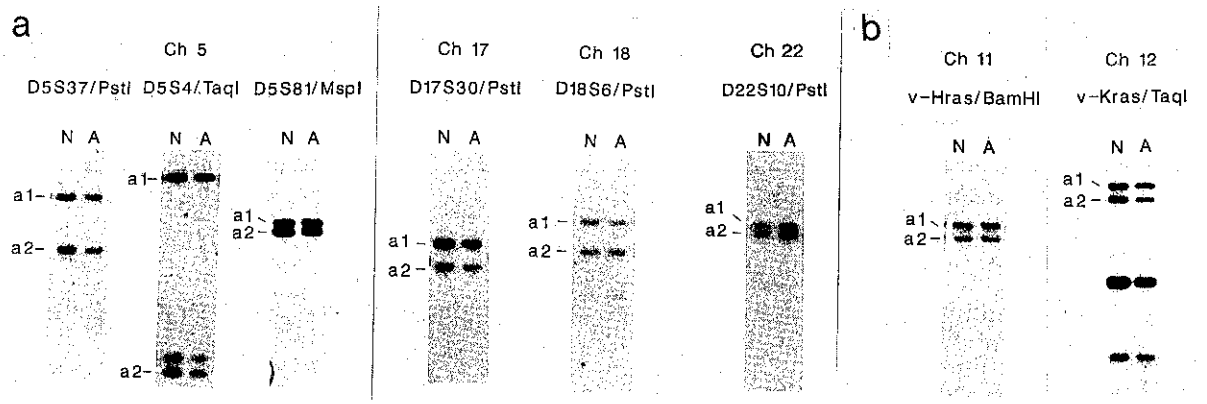

Fig. 4. Southern hybridization of chromosomes (Ch) 5, 17, 18, and 22, and c-H-*ras* (H*ras*) and c-K-*ras* (K*ras*) probes to normal tissue (N) and FPCK-1 cells (A). Restriction enzymes used are indicated adjacent to the probe. (a) Polymorphic DNA probes D5S37, D5S4, D5S81 were found on chromosome 5, D17S30 on chromosome 17p, D18S6 on chromosome 18p, and D22S10 on chromosome 22q. (b) No rearrangement or amplification of H-*ras* and K-*ras* genes is apparent. The viral *ras* genes were used to probe the cellular *ras* genes.

were subjected to examination for loss of heterozygosity on chromosomes 5, 17p, 18p, and 22q using specific polymorphic DNA markers (Fig. 4). The specific loci detected by the DNA probes have been reported to be lost in association with colon carcinomas of FPC patients at high frequency.[4, 10] Lymphocytes of the patient were the source of normal DNA. No loss of heterozygosity was observed (Fig. 4a). There was neither gene rearrangement nor gene amplification in H-*ras* and K-*ras* loci (Fig. 4b). Moreover, we could not detect any point mutation in codon 12 of the c-K-*ras* gene by a combined polymerase chain reaction and dot hybridization study utilizing specific 19-mer probes (data not shown).[10] At passage 19 (February, 1990) FPCK-1 cells were injected i.p. into 5 male BALB/c nude mice at the level of 5×10^6 per mouse; no tumor or nodule developed within a 7 months' observation period. Examination at the same passage did not reveal either rearrangement or amplification of *myc* and H-*ras* genes again. As of October, 1990, the culture has reached the 22nd passage in 3 years and is still growing retaining the same morphology and growth rate as at earlier passages.

Starting from 4 different adenomas of FPC patients, we have so far succeeded in establishing 3 immortal epithelial cell lines, including the present one, which have been consecutively cultured for more than two and a half years or to the 11th passage. In contrast, working with sporadic adenomas from non-FPC cases, only one long-lasting line out of 20 samples has been successfully passaged for more than four subcultures in 2 years.

Paraskeva and his colleagues also reported three human adenoma cell lines from FPC patients,[12, 13] one of which, designated PC/AA, established from a large tubular adenoma (3–4 cm), has been immortalized and further used for carcinogenesis experiments. The cell line has been passaged with 3T3 feeder cells more than twice as frequently as the present FPCK-1 cells, and a normal karyotype was conserved only in early passages (less than 50 passages), but the PC/AA already had the c-K-*ras* mutation at codon 12. Cells immortalized in culture with and without 3T3 feeders showed common abnormalities in chromosome 1. In the late passages (more than 50 passages) they became aneuploid and clonogenic but still did not grow either in soft agar or nude mice. Further treatment of the PC/AA cell line with sodium butyrate and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine resulted in *in vitro* progression, including development of severe chromosome abnormalities and tumorigenicity in nude mice.[16]

Unlike PC/AA cells, the present FPCK-1 cells grow very slowly (22 passages in 3 years) and have been remarkably stable without showing any chromosomal abnormalities, K-*ras* mutation, or loss of heterozygosity at specific loci suspected to be causative in colon carcinogenesis. According to Miyaki *et al.* polymorphic DNA probes revealed loss of heterozygosity and K-*ras* point mutation in advanced carcinomas but could barely detect them in relatively small adenomas with mild or moderate atypia.[10] We therefore consider that the FPCK-1 cell line may be more representative than the PC/AA cell line in retaining features of adenoma cells at a very early phase.

On the other hand Willson *et al.*[17] and Paraskeva *et al.*[18] reported establishment of 4 adenoma cell lines (2 lines in each report) from sporadic cases. While they did not grow either in soft agar or nude mice, in contrast to the cell lines from FPC patients (PC/AA and FPCK-1), those derived from sporadic adenoma demonstrated ex-

tensive chromosomal abnormalities except in one case. These data combined with our experience of difficulties in establishing sporadic adenoma cell lines would support the view that the intrinsic heterozygous mutant/wild type condition at the FPC gene may endow specific growth potential *in vitro* on the epithelial cells from FPC patients without further chromosomal abnormalities, and that sporadic adenoma cells require other or further chromosomal changes to survive *in vitro*.

In the case of FPCK-1 cells, the negative results regarding genetic alterations which have been reported to be associated with the development of colon carcinomas may suggest the existence of hitherto unknown mechanisms in the generation of FPC adenomas. The described cell line should prove useful for further investigation of the multistep nature of carcinogenesis in the colon of FPC patients.

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