ONCOLOGY

INDUCTION OF STROMAL CELL TRANSFORMATION IN XENOGRAFTS OF HUMAN COLONIC CARCINOMA

I. P. Bryzgalov, T. V. Yudicheva, S. A. Galetskii, Yu. N. Solov'ev, and E. S. Revazova

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To obtain xenogeneic tumors at the present time immunodeficient animals, mainly nude mice, are used, for in this way it is possible to create a system for determining the tumorigenicity of cells [2, 3] and the factors of the host which affect tumor development [11]. Reports on the possible induction of transformation of an animal's stromal cells in xenografts have been published [6, 8, 11]. This phenomenon is quite rare, and transformation can be discovered both during passage of tumors through an animal and when a graft is transferred into in vitro conditions [11].

In this paper we describe two cases of transformation of stromal fibroblasts from xenografts of human colonic carcinoma, discovered during serial passage of this tumor in nude mice and rats.

METHODS

Nude mice based on the BALB/c strain, aged 4-6 weeks, and nude noninbred rats aged 6-8 weeks, bred in our Institute, were used. Primary material from carcinoma of the human colon was obtained during operation and was transplanted subcutaneously into the left subscapular region of a nude mouse. The tumor was later subjected to serial passage. The 48th generation of this strain (kept in the frozen state) was transplanted subcutaneously into a nude rat, and this also was followed by serial passage. Cell cultures FM-7 and FR-7, of mouse and rat origin, respectively, were obtained from the xenografts. The two cell cultures were grown on MEM medium with a double set of amino acids and with 10% fetal calf serum at 37°C. Cell cultures in the logarithmic phase of growth, preincubated previously for 2 h in medium containing colchicine (Merck, Germany) in a concentration of 0.2 ng/ml, were used for cytogenetic analysis. The cells were subjected to hypotonic treatment with 0.56% potassium chloride solution for 35-40 min, after which they were fixed for 35-40 min in a mixture of acetic acid and methyl alcohol (1:3). Preparations were stained by the Giemsa method. The modal class of the chromosomes was determined in 100 metaphase plates. For the tumorigenicity test, cells ($5 \cdot 10^6$ in 0.3 ml) were inoculated subcutaneously in the subscapular region of nude mice. The tumors remained under observation for 6 weeks. Specimens for light microscopy were fixed in Carnoy's fluid, dehydrated, embedded in paraffin wax, and stained with hematoxylin and eosin and picrofuchsine, and by the PAS reaction. DNA for dot hybridization was isolated by lysis of the cells with 1% SDS solution followed by deproteinization with phenol/chloroform. Hybridization was carried out in the presence of 50% formamide with plasmid pBlur8, labeled with ^{32}P in the nick translation reaction, with specific activity of $1 \cdot 10^8$ cpm/μg DNA. Prehybridization, rinsing, and autoradiography were carried out as described previously [1].

RESULTS

Carcinoma of the human colon of strain RTK-7m was obtained by subcutaneous injection of material removed at operation into nude mice. By now the strain has gone through more than 100 passages. The characteristics of growth

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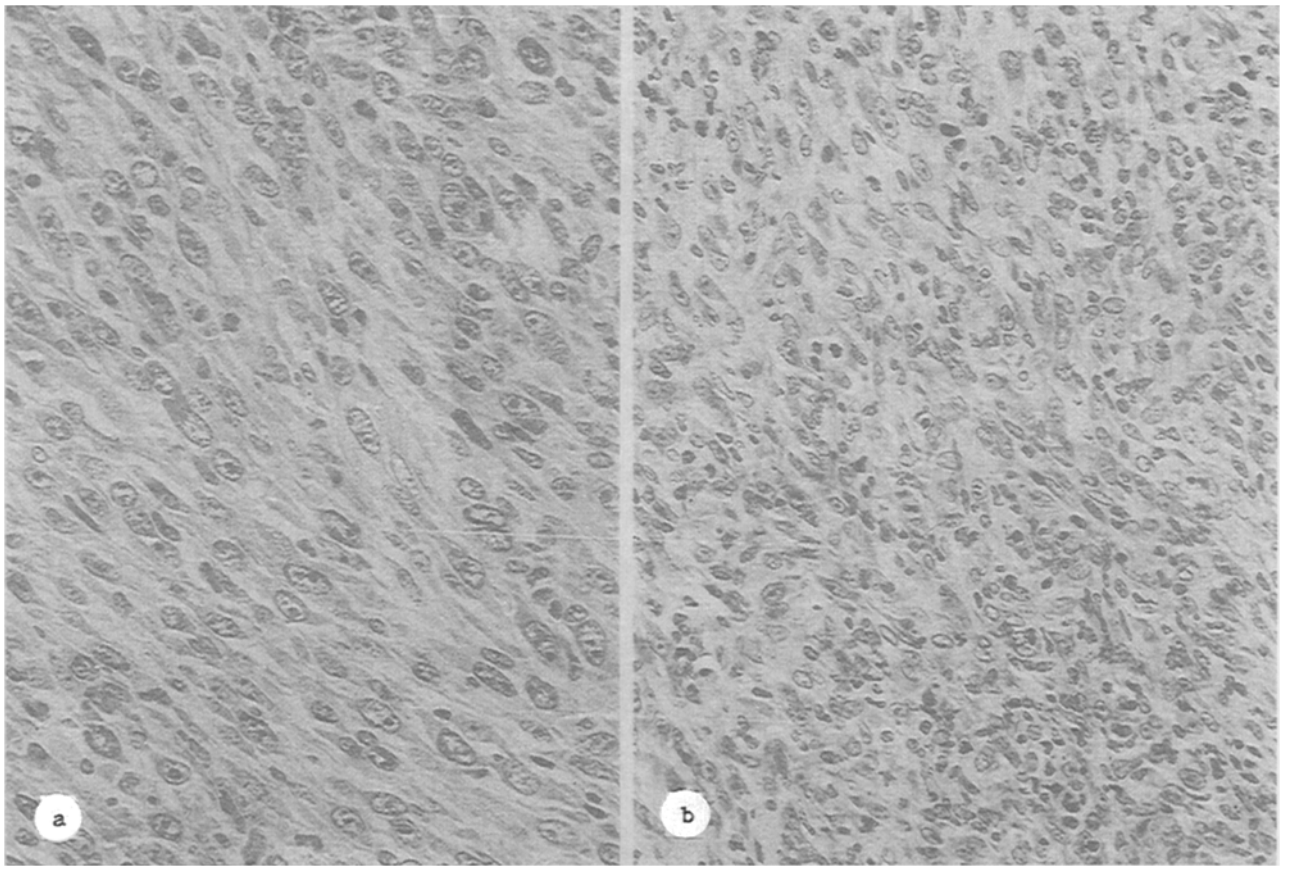

Fig. 1. Histologic picture of strains of human colonic carcinoma after passage through nude mice (a) (generation 41. Undifferentiated adenocarcinoma with transition into mucinous carcinoma. Stroma poorly developed. Hematoxylin-eosin, 250×) and nude rats (b) (generation 14. Undifferentiated adenocarcinoma. Hematoxylin-eosin, 400×).

of the strain and its morphological picture (adenocarcinoma; Fig. 1a) have remained stable until now.

To obtain a complex model, RTK-7m was taken at the 89th passage into tissue culture. However, beginning with the 5th passage, considerable predominance of fibroblast-like cells over epithelial was noted, and by the 16th passage, the latter were virtually absent. This cell culture (FM-7) grew to a compact monolayer (700,000-750,000 cells/25 cm^2 area of growth), and no foci of stratified growth were observed. To determine possible transformation, FM-7 cells at the 18th and 28th passages were injected subcutaneously into nude mice. All the animals (in groups of three mice) developed tumors after 3-4 weeks and they were characterized morphologically as fibrosarcomas (Fig. 2a). To determine the species to which the FM-7 cells belonged, karyologic analysis was carried out, revealing acrocentric chromosomes, unequivocal evidence of their murine genesis. The cell line does not have a clearly defined modal class, and cells containing a set of 38-39 chromosomes (14%) and 71-72 chromosomes (15%) predominate.

Considering that spontaneous transformation of mouse fibroblasts in cell culture could take place, although with a low degree of probability, in the first passages, to test the possible horizontal induction of stromal cell transformation we transplanted the 48th passage of RTK-7m subcutaneously into nude rats, and then subjected the tumor which appeared to serial passage. By now this strain (RTK-7k) has gone through more than 60 passages. The characteristics of growth and the morphological picture, similar to that of strain RTK-7m (adenocarcinoma; Fig. 1b), remained stable throughout the period of passage. At the 3rd passage strain RTK-7 was taken into culture. The cell culture thus obtained (FR-7), by the 6th passage, consisted of elongated fibroblast-like cells with round nuclei and large nucleoli, two to four per nucleus, and granular cytoplasm. The cell culture grew to a dense monolayer (800,000-850,000 cells/25 cm^2 growth

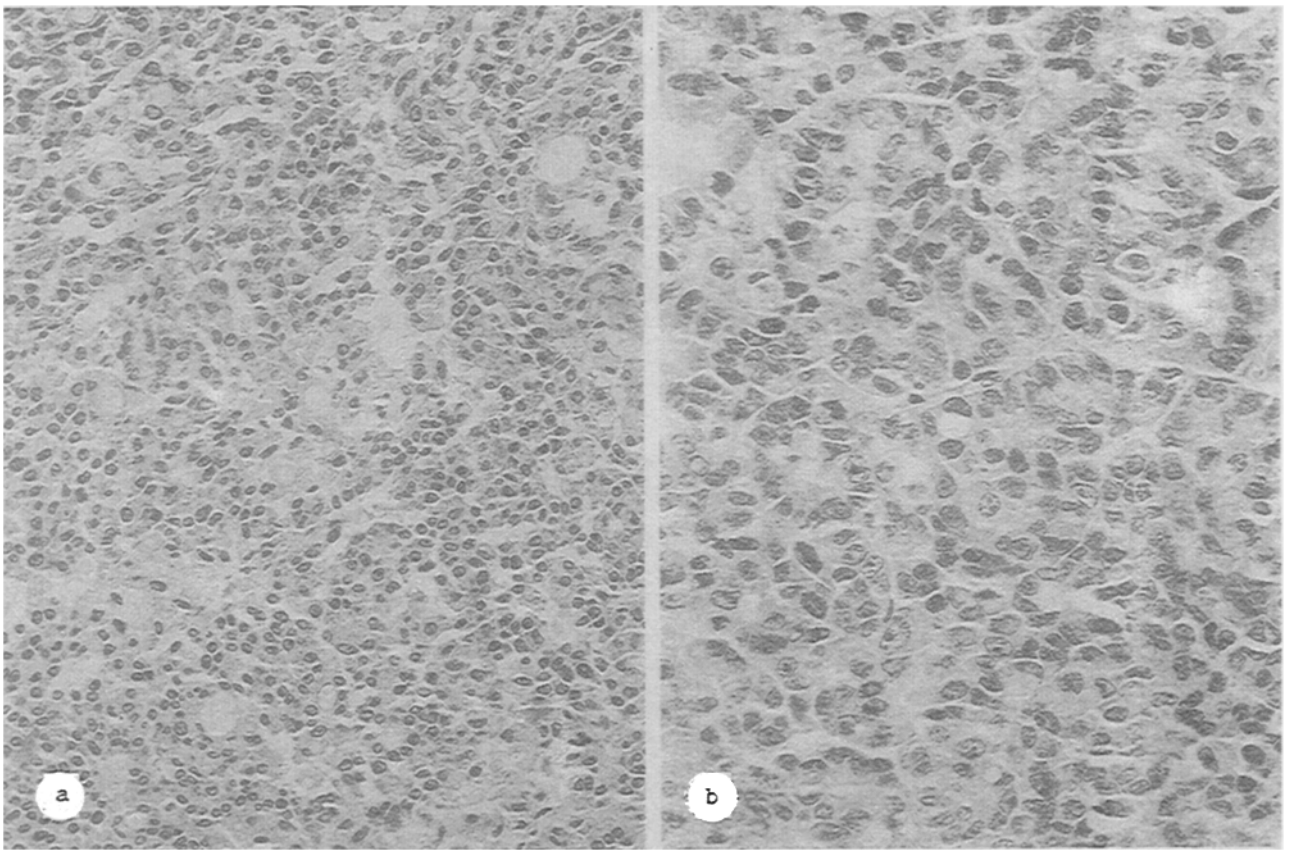

Fig. 2. Histologic picture of tumors formed after injection of FM-7 cells (a) (generation 18. Fibroblastic tumor with polymorphism of its cells. Picrofuchsine, 400×) and FR-7 cells (b) (generation 3. Proliferation of fibroblastic cells. Hematoxylin-eosin, 250×) into nude mice.

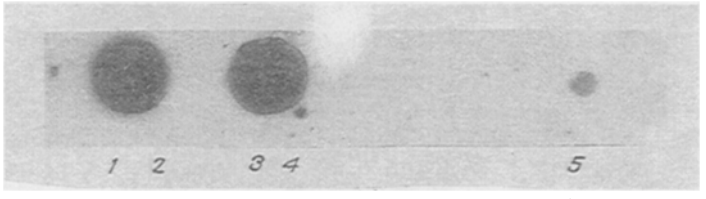

Fig. 3. Dot hybridization of ^{32}P-pBlur8 with DNA from rat cell lines FR-7 (2) and XC (4) human tumor strains HeLa (1) and RTK-7 (3), and nude mouse liver (5).

area); no foci of stratified growth could be seen at any time during more than 70 passages. To determine their tumorigenicity, FR-7 cells at the 3rd and 18th passages were transplanted subcutaneously into nude mice; this led to the appearance of slowly growing tumors of very firm consistency after 4-6 weeks. On histological investigation the morphological picture of a fibrosarcoma was discovered (Fig. 2b). Karyologic analysis revealed submetacentric and metacentric chromosomes, with the modal class of 40-42 chromosomes (51%). To detect the possible inclusion of human sequences into the genome of FR-7 cells the DNA from this cell line was tested for the presence of Alu-sequences. The results of dot hybridization with plasmid Blur-8 revealed the presence of hybridized sequences only in the positive controls: DNA from human tumors of the RTK-7k and HeLa strains. Autoradiography of DNA from

the liver of nude mice, from XC rat cells, and DNA from the FR-7 cell line revealed the virtual absence of a signal, proving the absence of sequences of human DNA in the genomes of the cells tested (Fig. 3). The results suggest that tumor cells from strain RTK-7 of carcinoma of the human colon can induce transformation of stromal fibroblasts in vivo; this phenomenon, moreover, is observed even after a relatively short period of passage of the xenograft (three passages).

The phenomenon of tumor-induced malignant transformation of normal tissue was discovered both in vivo and in vitro [11], but under these circumstances the mechanism of transformation still remains unclear, although several working hypotheses have been proposed [6, 8, 10].

One possible mechanism of transformation, which has been discussed in the literature, may be through activation of oncogenic viruses [4, 5, 7, 15]. However, the negative results of attempts to transform mouse fibroblasts by viral isolates in these cases [4, 13] and the absence of any association in other publications between malignant transformation of stromal fibroblasts of xenografts and the presence of retroviruses and/or the discovery of reverse transcriptase activity, suggest that this phenomenon is an accompanying factor.

Malignant transformation may also be connected with epigenetic factors [11, 12]. The transforming growth factors produced by many human tumors [14], in combination with intercellular interactions, probably determine the appearance of the transformed phenotype of stromal fibroblasts in xenografts of human tumors [11].

Thus human tumor cells of the RTK-7 strain can induce malignant transformation of stromal fibroblasts in nude mice and rats. The process of horizontal oncogenesis is not determined by primary transfection of DNA and by intercellular fusion. This model is interesting for further study.

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