SM/J H-2 haplotype H-2$^v$ which has been placed on the genetic background of strain C57BL/10Sn by repeated backcrossing. The only other known chromosome 17 variants between these strains are those addressed in this study.
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# Malignant Potential of Murine Stromal Cells After Transplantation of Human Tumors into Nude Mice

Abstract. *Human malignant cancer tumors grafted into nude mice produce tumors containing both human cancer cells and the host's stromal cells. After short-term propagation of these tumors in vitro, the murine mesenchymal cells appear transformed and are tumorigenic in nude mice. However, established human cancer cell lines fail to similarly alter adjacent murine stromal cells when used to produce tumors in nude mice. These experiments suggest that cancer cells may recruit normal cells to become malignant, qualifying the view of the clonal (unicellular) origin of cancer.*

The development of a malignant tumor is generally attributed to the selection and growth of a single clone originating in one or more mutational events (*1*). The heterogeneity of cells in a tumor can be explained by the subsequent selection of mutated variants, especially among a karyologically unstable population of cells (*1*). This heterogeneity is particularly pronounced in tumors composed of histogenetically mixed cell types, which have been recorded since the beginnings of histopathology (*2*).

Another explanation for the occurrence of mixed cell types and different cell populations in a tumor is the horizontal induction of malignancy in differentiated normal host cells by adjacent malignant cells, a process that may be mediated by oncogenic viruses, cell products, cell fusion, or as yet unknown mechanisms. This would imply that neoplasia is a continuing process whereby cancer cells transfer the malignant genotype to nonmalignant cells. The experiments described here support this hypothesis. They involve the transplantation of human tumors into nude mice, the cultivation of these tumors in vitro, the separation of murine stromal cells showing evidence of transformation, and the production of murine fibrosarcomas by the stromal cells (*3*).

A freshly excised adenocarcinoma of the colon of a 53-year-old man was minced and suspended in 0.9 percent sterile NaCl (50 percent tumor cell suspension, weight to volume) containing penicillin (200 U/ml) and streptomycin (50 μg/ml). One milliliter of this suspension was injected subcutaneously into the flanks of each of two nude mice (*nu/nu*-BALB/c). There were tumors in both animals after about 2 weeks, and after 4 weeks a tumor measuring 10 by 23 mm was excised from one mouse and used for tissue culture, histological evaluation, and retransplantation into three other mice. The microscopic appearance of the tumor grafts was similar to that of the original tumor (Fig. 1, a to c). The portion used for in vitro propagation was washed in Hanks balanced salt solution (Gibco) containing streptomycin and penicillin at five times the previous concentration. The minced tumor was incubated with 0.25 percent trypsin (Gibco) for 1.5 hours and the resulting cell suspension was cultured in a 75-cm$^2$ plastic flask containing Liebovitz L-15 medium and 15 percent fetal bovine serum, penicillin (200 U/ml), and streptomycin (50 μg/ml). Twenty-four hours later, the unattached cells were poured off, centrifuged, and cultured in a second 75-cm$^2$ flask. After 3 weeks colonies of epithelioid tumor cells were observed surrounded by transformed fibroblastoid cells showing loss of the inhibition that normal cells have upon contact in monolayer culture (Fig. 1d).

The second-passage transplants grew in all three mice. After 2 weeks a tumor measuring 16 by 15 mm (Fig. 1c) was excised for propagation in vitro and in vivo. After 24 hours the unattached cells in culture were removed and subcultured in another flask until confluence was achieved (2 weeks) (Fig. 1e). The fibroblastoid cells demonstrated an ability to detach and grow in suspension (anchorage independence), could be subcultured in continuous passage, and had a murine karyotype and a modal chromosome number of 77 (Fig. 2). Subsequent inoculation of nude mice with $1.0 \times 10^7$ cells resulted in fibrosarcomas (Fig. 1f), confirming the tumorigenic potential of the

Table 1. Findings for nude mouse passages of the GW-985 human colon adenocarcinoma in vitro; N.A., not available.

| Number of passages | Earliest transformation in vitro (weeks) | Morphology | | Modal chromosome number | |
|---|---|---|---|---|---|
| | | Epithelioid | Fibroblastoid | Human | Murine |
| 1 | 3 | Yes | Yes | N.A. | 77 |
| 2 | 2 | Yes | Yes | N.A. | 66; 69 |
| 5 | N.A. | Yes | No | 50 | N.A. |
| 17 | 10 | Yes | Yes | N.A. | 76 |
| 19 | 8 | Yes | Yes | N.A. | N.A. |
| 20 | 5 | Yes | Yes | N.A. | N.A. |

Table 2. Results of in vitro cultivation of human tumors that were transplanted into nude mice. The number of passages in vivo is given by the number in parentheses; N.A., not available.

| Tumor number | Locus of adenocarcinoma | Earliest in vivo passage explanted (weeks) | Earliest appearance of transformation in vitro (weeks) | Modal chromosome number of tumor explants | |
|---|---|---|---|---|---|
| | | | | Human | Murine |
| GW-869 | Colon | 6 | 2 | 50 (12) | 76 (10) |
| GW-870 | Ovary | 5 | <1 | N.A. | 60 to 70 (18) |
| GW-875 | Colon | 5 | <1 | 85; > 100 (1) | 70 to 74 (7) |
| GW-927 | Ovary | 1 | 3 | N.A. | 67 (1) |
| GW-985 | Colon | 1 | 3 | 50 (5) | 77 (1) |


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transformed murine fibroblastoid cells. These fibrosarcomas had an aneuploid murine karyotype.

In vitro propagation of different transplant generations of the human colon adenocarcinoma resulted in a similar development of distinct epithelioid and fibroblastoid cell populations, the latter also showing properties of transformation in vitro and tumorigenicity in vivo. Even after 20 in vivo passages of the human tumor over 14 months, it was still possible to recover transformed murine fibroblasts from the tumor transplants when the human tumor grafts were explanted (Table 1). The epithelioid tumor cells continued to produce tumors in nude mice, tumors histopathologically resembling the original adenocarcinoma. However, on one occasion (fifth transplant generation), malignant murine fibroblasts could not be recovered.

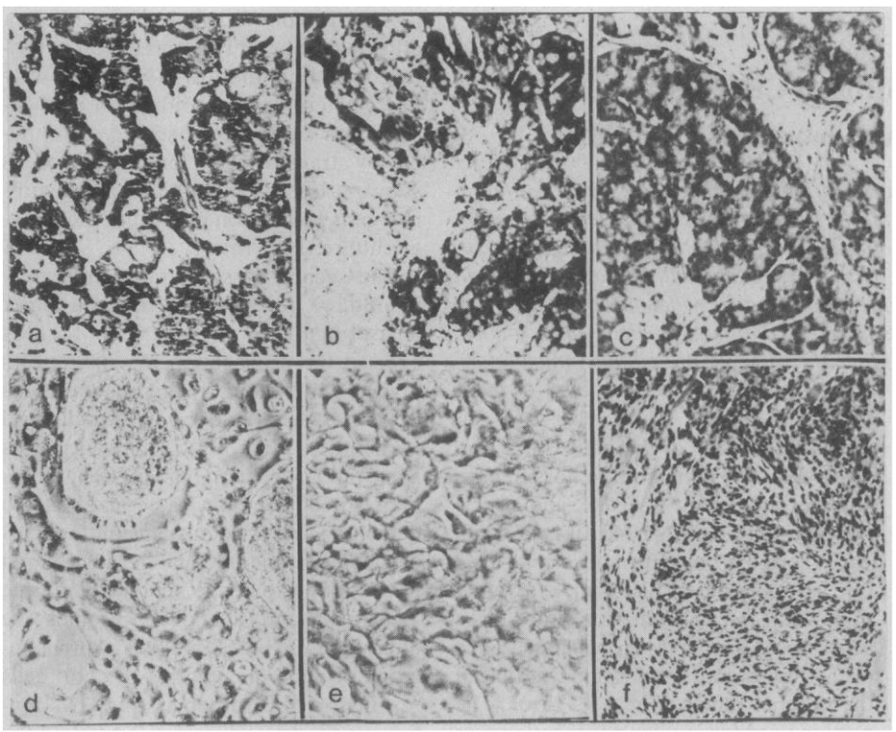


Fig. 1. (a) Microscopic morphology of the human tumor specimen used in the nude mouse transplantation experiments. Note the areas of highly differentiated colonic adenocarcinoma (hematoxylin and eosin; original magnification, ×125). (b and c) Histology of the first (b) and second (c) transplant generations of human colon adenocarcinoma (GW-985), indicating similarity between the original tumor and the xenografts (hematoxylin and eosin; original magnification, ×125). (d) Appearance of the cells of the first nude mouse tumor transplant 3 weeks after cultivation, showing colonies of colonic tumor cells surrounded by transformed fibroblastoid cells (original magnification, ×100). (e) Two-week-old culture of second-passage nude mouse tumor showing confluent growth of transformed fibroblastoid cells (original magnification, ×100). (f) Microscopic morphology of tumor resulting from inoculating a nude mouse with transformed fibroblastoid cells. Note the mesenchymal nature of the malignant neoplasm (hematoxylin and eosin; original magnification, ×125).

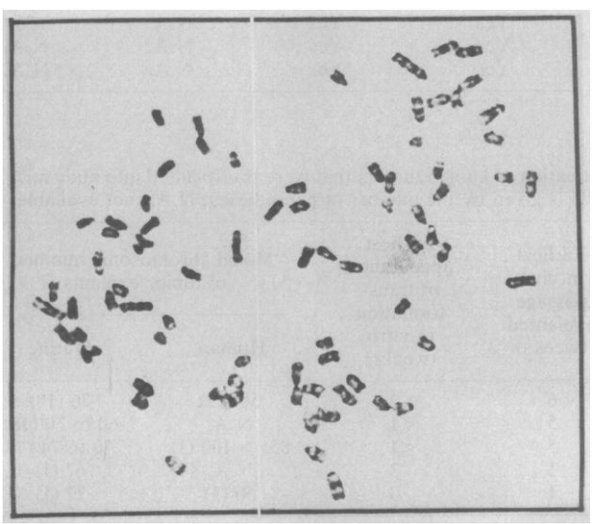

Fig. 2. Typical metaphase spread of chromosomes from a transformed fibroblastoid cell derived from the human tumor transplant (GW-985) after cultivation in vitro. The cell has 79 typically murine chromosomes, including two metacentric-appearing chromosomes that are the result of centric fusions (Giemsa, original magnification, ×1000).

To assess the generality of this event, ten different human tumors were serially passaged in nude mice by injection of primary human cancer cells. These tumors included such diverse histopathological types as transitional cell carcinoma of the urinary bladder; renal carcinoma; adenocarcinomas of the ovary, endometrium, adrenal cortex, and colon; and squamous cell carcinoma of the uterine cervix. A population of malignant stromal cells derived from the human tumor grafts was then separated in culture and established as fibrosarcomas in nude mice (Table 2). The earliest passage of a primary human tumor in nude mice resulting in the isolation of malignant fibroblasts was the first transplant generation (GW-927 and GW-985), and the longest in vivo propagation of human tumors showing this phenomenon was 14 months (20 passages of GW-985) (Table 1). The earliest appearance of transformed fibroblastoid cells in vitro after explanation of the nude mouse tumor grafts was less than 1 week (GW-870 and GW-875) (Table 2). These results were reproducible on several occasions. After separation in vitro, the human and murine tumor cell populations showed aneuploid karyotypes and remained individually tumorigenic in nude mice.

The shortest in vitro cultivation of the tumor stromal cells before evidence of transformation was observed was less than 1 week in two cases (GW-870, an adenocarcinoma of the ovary, and GW-875, an adenocarcinoma of the colon). Thus it is unlikely that the transformation of these fibroblasts was a spontaneous event in culture. Moreover, lung fibroblasts of nude mice that were growing under similar conditions for up to 14 weeks did not show evidence of transformation in vitro or tumorigenicity in vivo. Nevertheless, it can be argued that the ability of mouse connective tissue cells to undergo so-called spontaneous transformation in culture is accelerated after their interaction with human cancer cells.

We tried to determine whether murine fibroblasts with a malignant potential are present in all human tumors growing in nude mice by repeating these experiments with established human cancer cell lines. When three such cell lines [LS-174T (colonic adenocarcinoma) and E14/115 and Sh-oat (bronchogenic carcinomas)] were inoculated into nude mice

in separate experiments, they produced tumors consistent with their original histopathology. Cultures of these transplants by methods identical to those used for the primary human tumor transplants did not result in propagation of the transformed fibroblastoid cells despite several attempts. It thus appears that stromal cell oncogenicity in human tumor transplants is restricted to human tumors that are first established in nude mice. We cannot rule out the possibility that this could result from the interaction between mouse stromal cells and the human stromal cells present in the original transplants, since cultured cancer cells (lacking human stroma) did not give the same results. However, different transplant generations of human tumors devoid of human stroma showed murine stomal cell oncogenicity in nude mice (Tables 1 and 2).

A tumor's stroma has generally been considered to be a supportive structure nourishing the growing neoplasm. Less clear has been whether the tumor has genotypic effects on the stroma, or vice versa, although sarcomatous transformation of the connective tissue stroma of mouse mammary adenocarcinoma has been reported repeatedly since 1905 (*4*). The question of whether malignant cells can transfer malignancy to other cells has been addressed previously (*5*); that such transfer occurs is supported by our observations. Human tumor xenografts in nude mice were found to contain murine host cells which, after short-term culture in vitro, showed evidence of malignant transformation. The transformed cells were then able to produce sarcomas in other nude mice. This has been confirmed by transplanting a human embryonal carcinoma into nude mice; after the human tumor graft was cultured in vitro, the overgrowth of murine cells showed signs of malignancy (*6*). However, these investigations failed to produce tumors in mice with the transformed murine cells. Furthermore, a similar finding was made when a human melanoma was transplanted into nude mice (*6*).

Grafts of human tumor cells to immunodeficient animals can produce tumors of host origin (*7*). We therefore presume that, in our experiments, in vivo oncogenesis of mouse stromal cells in the human tumor grafts occurred but that these cells were unable to grow alongside the human tumor cells as a sarcoma until the malignant stromal cells were propagated in vitro, separated, and inoculated into appropriate recipients. Perhaps oncogenic viruses were activated in this process, since Gautsch *et al.* (*8*) recently found that 1 of 20 human tumors transplanted and passaged in nude mice was associated with the induction of endogenous murine leukemia virus. However, we had one of our murine sarcomas and three human tumor transplants in nude mice tested for virus, and the results were negative (*9*).

The alterations in the murine stromal cells isolated from the human grafts may have occurred in vitro, but this is unlikely because of the very short duration in culture before transformed cells were observed and because such results were not obtained when human tumor grafts made from established human tumor cell lines were cultivated in vitro. Nevertheless, we cannot exclude the possibility that the human tumor cells accelerated the in vitro transformation of murine stromal cells. Another possibility is that the malignant murine stromal cells resulted from in vitro or in vivo hybridization of the human tumor and mouse stromal cells, with subsequent loss of the human genome and retention of the malignant genotype. Indeed, somatic cell fusion in vivo has been reported (*10*), but not involving tumors in nude mice. Although we do not have any evidence to support this explanation, we must conclude that some form of cell-cell interaction results in the alteration and ultimate oncogenesis of normal murine stromal cells contained in human cancer xenografts. Interestingly, this event was only observed with human tumors grafted directly from patient specimens, and not with established human cancer cell lines.

We realize that extrapolation of these findings to human and other primary cancers is limited by our experimental system. Nevertheless, the widespread use of nude mice in cancer research warrants the caution that, under the conditions described here, nude mouse cells can become malignant.

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## Eicosapentaenoic and Arachidonic Acids from *Phytophthora infestans* Elicit Fungitoxic Sesquiterpenes in the Potato

Abstract. *Mycelial extracts from* Phytophthora infestans *caused necrosis and elicited the accumulation of antimicrobial stress metabolites in potato tubers. A portion of the material with elicitor activity could be extracted from the mycelium by a mixture of chloroform and methanol. The most active elicitors of stress metabolites in these extracts were eicosapentaenoic and arachidonic acids. These fatty acids were found in either free or esterified form in all active fractions of the mycelial extracts.*

When plants interact with parasites, some resistant cultivars respond hypersensitively to incompatible races of pathogens by accumulating antimicrobial stress metabolites (*1*). These stress metabolites, which may be a defense mechanism against disease, are elicited by phytotoxic compounds that are produced by fungi and bacteria (*2*). Incompatible races of the fungus *Phytophthora infestans* (Mont.) de Bary, which causes late blight disease in the potato, elicit the accumulation in tuber tissue of rishitin and lubimin as well as other fungitoxic


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