Sarcomatous Transformation of the Stroma of Mammary Carcinomas That Stimulated Fibroblastic Growth in Vitro*

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INTRODUCTION

Sarcomatous transformation of the stroma of transplantable mouse carcinomas was first described by Ehrlich and Apolant (5). The following year a case was reported by Loeb (13). Subsequently the phenomenon was observed to occur twice in a strain of mammary carcinoma maintained in these laboratories, and a preliminary note on the subject was published by Bashford, Murray, and Haaland (4). Later a detailed histological investigation was carried out by Haaland, whose paper (8) effectively dealt with criticisms that had been raised as to the reality of sarcomatous transformations. “All evidence,” he wrote, “seems to speak for a gradual process by which apparently normal connective tissue cells evolve into sarcomatous elements, endowed with altered biological qualities.” Since this was written other cases have been reported from time to time, but sarcomatous change has been regarded in the past as a “rare occurrence.” Thus in a general review of the tumors studied in these laboratories up to 1911, Bashford (2) commented, “the carcinomata which induce sarcomatous transformation of their stroma are of rare occurrence.” This conclusion was based on observations upon more than 650 primary tumors and 85 strains of propagated tumors of various histological types. Sarcomatous transformation was observed in only four strains. “In two the change supervened during transplantation. In the other two the change took place in the primarily affected animal.”

Of the various suggestions advanced as to the cause of sarcomatous change it is of interest to recall that Apolant and Ehrlich (1) attributed it to “a stimulating influence proceeding from the carcinomatous cells, which in a certain phase of their development determines the sarcomatous transformation of the connective tissue scaffolding of the tumor.” Now, 38 years after this was written, we have been able to adduce evidence that mammary carcinomas that had “a stimulating influence” upon fibroblasts in tissue cultures have undergone sarcomatous change. In our previous paper (15) we demonstrated stimulation of fibroblastic growth in vitro by carcinomas of high mammary cancer strain mice. The present communication is concerned with the sarcomatous changes occurring in those tumors that were maintained by transplantation. Contrary to the experience of the earlier investigators, who worked with hybrid mice of unknown genetic constitution, our experiments indicate that sarcomatous change is a common occurrence in mice of high mammary cancer strains.

It should be pointed out that the evidence for the occurrence of sarcomatous transformation has not been universally accepted as conclusive, based as it is on the histological interpretation of transitional stages. An alternative explanation negates the idea of neoplastic changes in the stroma, and attributes what appear to be sarcoma cells to morphologically altered epithelial cells (spindle cell metaplasia). “Such transformations are relatively common in the early or advanced stages of many human tumors, and especially in recurrences after operation, and this fact establishes a probability that a similar change in mouse tumors has a similar significance.” So wrote Ewing (6), who concluded: “Since such an interpretation is at least admissible, it may be urged that further evidence is required before the sarcomatous transformation of mouse carcinoma can be accepted as proved.”

Our purpose in this paper is to furnish further evi-
Experiments

Four different mammary carcinomas that arose spontaneously in mice of the Strong A strain, and 2 that originated in mice of the RIII strain of Dobrovolkskaia-Zavadkskaia, were transplanted serially by subcutaneous grafts so as to have available a supply of tumors for testing therapeutic substances. At each transplantation a fragment of tumor was fixed for histological examination, and tumors of different generations were also grown in tissue cultures. It was observed that both the histological sections and the morphology of the culture exhibited a progressive change from carcinoma to sarcoma. The histological changes occurring during sarcomatous transformation of the stroma of carcinomas were very fully described by Haaland (8). We are able to endorse his statement that "neither in the primary tumour nor in the earlier generations of the strains leading up to sarcoma development do we find any stroma elements with peculiar characters"; and that "there is a definite stage before the appearance of the sarcoma in which the stroma of the tumour has become more abundant and cellular." In addition to the 6 tumors that we have had under observation during the sarcomatous transformation, 5 others of high mammary cancer strains have begun to exhibit this presarcomatous change.

That the 6 tumors studied by us have definitely undergone sarcomatous transformation, and not merely a spindle cell metaplasia, is most lucidly demonstrated by the tissue culture experiments. There is universal agreement that carcinomas grow typically as flat sheets, or as pavement-like growths of closely adherent cells, while in sarcoma cultures the cells are spindle-shaped or polymorphous and separated to varying degrees. (Lambert and Hanes [10]; subsequent work reviewed by Fischer [7], Ludford [14], and Levi [11]). A 4 day old coverglass culture of a primary tumor of a Strong A mouse is shown in Fig. 1. The carcinoma cells spread themselves out with facility, so that the living culture presents the appearance of a rounded, translucent membrane. The lighter area between the explant and the periphery of the growth in the photo-

Figure 1. A sarcomatous growth from a tumor of the 19th generation transplant is shown in Fig. 4. This is comprised of numerous separate large spindle-shaped to polymorphous cells, again with very considerable numbers of cells of the monocyte-macrophage type. These extend out into the medium far beyond the outermost of the sarcoma cells.

This particular tumor was unique amongst our 6 cases in that large atypical cells were first observed in cultures from a tumor of the 11th generation, approximately 9 months after the first transplantation. Since these cells exhibited the same cytological characters as have persisted throughout subsequent generations and now comprise the greater part of the tumor, we consider their appearance as indicative of the sarcomatous transformation. But they did not rapidly overgrow and replace the carcinoma cells in vitro, which is what occurred with the other 5 tumors. Instead, they grew along with the carcinoma cells, constituting a carcinosarcoma. When small fragments of a tumor were explanted, 3 types of cultures resulted; (a) sheets of carcinoma cells, (b) growths of large spindle cells with innumerable monocytes and macrophages, and (c) mixtures of these two. Sometimes there was a sheet growth on one side of a culture and spindle cell
Fig. 1.—Four day old culture of a mammary carcinoma of a Strong A mouse (primary tumor).

Fig. 2.—Five day old culture of the sarcoma that originated as a result of sarcomatous transformation of the stroma during transplantation of the mammary carcinoma shown in Fig. 1, at the same magnification.
FIG. 3.—Three day old culture of another mammary carcinoma of a Strong A mouse (eighth generation transplant).

FIG. 4.—Three day old culture of the sarcoma resulting from sarcomatous transformation of the stroma of the mammary carcinoma illustrated in Fig. 3, at the same magnification.
growth on the other side. Most frequently there was a considerable outgrowth of sarcoma cells and monocytes and macrophages, and a residual compact growth of carcinoma cells within the explant. In the culture illustrated in Fig. 4 there are considerable numbers of carcinoma cells in the explant. Though there are well defined cytological differences between the carcinoma cells and the sarcoma cells, in addition to their shape and mode of growth, it is not intended to enter here into a discussion of the detailed cytology of these tumors; this will be reported in a later paper.

Sections of this tumor during its carcinomasarcomatous phase exhibit large compact groups of carcinoma cells, in an extensive stroma consisting of hyperchromatic cells varying notably in size, with a considerable admixture of cells of the monocyte-macrophage type. Sections of the 20th generation still show areas of carcinoma and sarcoma, but suggest that the carcinomatous elements will eventually be eliminated.

With the other 5 tumors the sarcomatous change was completed very rapidly; as far as we are able to determine, within a single generation, though our methods for distinguishing between fibroblasts, presarcomatous fibroblasts, and sarcoma cells lack the precision necessary for an unequivocal decision. Cultures prepared from tumors, the sections of which exhibited the presarcomatous changes of a more abundant and cellular stroma, were characterized by a considerable growth of fibroblasts with macrophages, usually extending outwards beyond the sheet growth of carcinoma cells. The fibroblasts that first appeared differed considerably from the sarcoma cells that grew ultimately. Although it is not always possible to distinguish with certainty whether any one cell is an abnormal fibroblast or a definite sarcoma cell, yet, as Lewis (12) has pointed out, sarcoma cells in general are characterized by an "increase in size of cell and nucleus, increase in density of cytoplasm, increase in number and decrease in size of the mitochondria, increase in the amount of nucleolar material, increase in thickness of the nuclear membrane and the granular condition of the nucleoplasm."

Were our evidence for sarcomatous transformation of these mammary carcinomas limited to differences in cellular morphology and in their growth patterns in vitro it would amount to little more than an amplification of the histopathological data. But we are able to add to it the demonstration of a difference in the biological properties of the cells that exhibit the different morphological features. In a previous paper (15), we adduced evidence that cultures of mammary carcinomas from high cancer strain mice stimulate the growth of fibroblasts, while sarcomas induced by carcinogenic hydrocarbons inhibit fibroblastic growth. We have employed the same technic to determine the influence on fibroblastic growth of these tumors when they are growing as typical carcinomas (Figs. 1 and 3) and as sarcomas (Figs. 2 and 4).

Mouse fibroblasts were grown between two explants of carcinoma and later, when the tumor exhibited the morphological indications of complete sarcomatous change, between two explants of this tumor. Growth of fibroblasts in the presence of the 2 tumors was compared with the growth of fibroblasts in the presence of other cultures of the same fibroblasts. The technic of cultivation and the method of measuring fibroblastic growth was the same as previously described. The carcinomas exhibited fibroblastic growth stimulation of the same order as described in our former paper. The tumors derived from their stromas, which were diagnosed histologically as sarcomas, inhibited fibroblastic growth to varying degrees, as had the sarcomas induced by carcinogenic hydrocarbons, with which our previous experiments were conducted. Figs. 5 and 6 demonstrate the difference in the growth of fibroblasts in the presence of a transplanted RIII carcinoma (Fig. 5) and of the spindle cell sarcoma to which it gave origin (Fig. 6). Both were photographed at the same magnification. Fig. 5 represents the best growth of fibroblasts obtained in this particular experiment after 7 days. Since fibroblastic growth in untreated cultures invariably ceases sooner in the presence of sarcoma than in the presence of carcinoma, the culture shown in Fig. 6 of the series, was fixed after 5 days, as it had begun to exhibit early indications of cellular degeneration. The 2 cultures are thus not strictly comparable, but in spite of the discrepancy in age of these 2 fibroblast cultures it is obvious that they have been subjected to different types of action, which have been determined by the different biological properties of the cells responsible.

DISCUSSION

As the foregoing observations indicate, tumors that from histological evidence are considered to have originated from fibroblasts of the stroma of mammary carcinomas are indeed true sarcomas. Their sarcomatous nature is indicated in cultures by their growth pattern and general cellular morphology, which resembles that of fibroblasts rather than of epithelial cells; by their high content of cells of the monocyte-macrophage type; and by their property of inhibiting the growth of fibroblasts. That the first 6 tumors with which we commenced this work should all have undergone sarcomatous transformation, apart from others now showing presarcomatous changes, implies that such transformations are a common occurrence in high cancer strains, at least in the Strong A and RIII strains. The selection of these tumors for transplantation in the first instance was purely arbitrary. They
Fig. 5.—Culture of embryonic mouse fibroblasts grown between two explants of a mammary carcinoma from an RIII mouse (third generation transplant). Seven day old culture.

Fig. 6.—Culture of embryonic mouse fibroblasts grown between 2 explants of the sarcoma that originated by sarcomatous transformation of the stroma of the RIII tumor that stimulated the growth of fibroblasts illustrated in Fig. 5. Five day old culture, same magnification as Fig. 5.
were taken at random from our stocks of inbred mice as being of sufficient size to yield abundant tissue for transplantation into a large number of mice. The method of transplantation varied from time to time. When large numbers of mice were to be inoculated, tumors were minced to a pulp with scissors and injected with a syringe, but when transplantation was limited to a few mice, fragments of tissue, about 2 mm. in diameter, were inoculated with a trochar. We have no evidence that either method of transplantation specifically influenced the induction of the sarcomatous change.

In seeking an explanation for the frequency of the sarcomatous change certain factors demand special consideration. Particularly significant is the fact that all our mammary carcinomas that underwent this change had been found to be powerful stimulants of fibroblastic growth in vitro, as previously reported. This can mean only that something was liberated by the growing carcinoma cells that excited the fibroblasts to increased growth. We have no definite proof that a similar stimulation was operative in vivo, but its occurrence was indicated by the more abundant and cellular character of the stroma that preceded the sarcomatous changes. As was pointed out in our previous paper the carcinomas that stimulate fibroblastic growth most conspicuously are the mammary carcinomas of high cancer strain mice, and these are the tumors that have exhibited sarcomatous transformation of the stroma. In their etiology the "mammary tumor inciter" of Bittner is of fundamental importance, but we have as yet no evidence whether or not it is concerned in the sarcomatous transformation. Experiments directed towards elucidation of this aspect of the problem are still in progress.

That the special genetic constitution of the high mammary cancer strains may be an important factor seems highly probable, but here again our evidence is as yet equivocal and will be left for discussion to a later communication. Attention should be directed, however, to the difference between our transplantation experiments with inbred strains of mice and the work of the earlier investigators, who used mice of mixed genetic constitution. Thirty-nine years ago Bashford, Murray, and Cramer (3) published the first account of the "source of the constituent elements of new stroma" after 3 days, and as previously reported. They confirmed the earlier observations of Jensen (9), that the new tumor parenchyma is derived solely from that introduced, and demonstrated that the "stroma and vascular structures are merely a reaction on the part of the successive hosts, whereby the parenchyma is nourished and supported by an artificial circulation renewed from time to time." They described the stroma of carcinoma grafts as beginning to degenerate 24 hours after inoculation, as being "extremely degenerated in all its elements" after 3 days, and as reaching "the last stage of degeneration" after 4 days.

In his study of sarcomatous transformation Haaland (8) was led to consider the possibility of fibroblasts of the stroma surviving transplantation, and their "survival after repeated subtransplantation into successive hosts" was suggested as a contributing factor to the induction of malignancy. He contributed corroborative evidence about which he wrote: "In examining early stages of tumors in this presarcomatous stage with abundant and cellular stroma, we found in single cases strong evidence of connective-tissue elements being transplantable, before any sarcomatous change shows itself histologically. We have seen the difficulties in the way of deciding when this transplantability of individual stroma elements has appeared for the first time, and the possibility remains that the transplantation of individual stroma elements may go further back than can be proved by our methods." It might be expected that when a tumor is transplanted into mice of the same genetic constitution, the likelihood of stroma elements surviving would be much enhanced, since the cells of the transplanted tumor stroma are then homozygous with those of the new hosts. We have investigated this possibility and, without entering into the details of our findings here, it is pertinent to point out that our evidence indicates that stromal cells from even the first generation transplant of a mammary carcinoma appear to survive when transplanted into a new host. Stromal cells at the periphery of such a graft do not undergo the early degenerative changes that Bashford, Murray, and Cramer described, but it is not possible to be absolutely certain that they survive to proliferate indefinitely, owing to the difficulty of distinguishing between these cells and others that invade the graft in bringing about the new vascularization.

Of the various factors, then, that might be responsible for the frequency of sarcomatous transformation during the transplantation of mammary carcinomas of high cancer strain mice, our present evidence emphasizes particularly the significance of the stimulation of fibroblastic growth by the carcinoma cells, and the greater survival of stromal cells when transplanted because they are homozygous with the cells of their new host.

SUMMARY

1. Sarcomatous transformation of the stroma is a common occurrence during the transplantation of mammary carcinomas of high cancer strain mice.

2. The histological evidence of sarcomatous change is confirmed by study of the growth characteristics of tumors in vitro before and after they have undergone transformation.
3. In tissue cultures mammary carcinomas exhibit the typical epithelial growth pattern, with few cells of the monocyte-macrophage type, and stimulate fibroblastic growth (Figs. 1, 3, and 5).

4. The sarcomatous nature of the transformed tumors is indicated by their growth pattern and general cellular morphology, resembling fibroblasts; by their high content of cells of the monocyte-macrophage type; and by their inhibiting fibroblastic growth (Figs. 2, 4, and 6).

5. Of the factors responsible for the frequency of sarcomatous change in the high mammary cancer strains, special significance is attributed to: (a) the considerable stimulation of fibroblastic growth by the carcinoma cells; and (b) stromal cells surviving transplantation because the cells of the graft are homozygous with those of the new host.

REFERENCES


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