Histological and Biochemical Studies of a Transplantable Rat Chondrosarcoma


Connective Tissue Section, Laboratory of Development Biology and Anomalies, National Institute of Dental Research, NIH, Bethesda, Maryland 20205 (D. B., L. D. de L., L. P.); Department of Medicine, and the Institute of Dental Research, University of Alabama, Birmingham, Alabama 35294 (S. G.); Hoffmann-LaRoche Inc., Nutley, New Jersey 07110 (R. L. S.); and Department of Pathology, University of California, San Francisco, San Francisco, California 94143 (R. S.).

ABSTRACT

The Swarm rat chondrosarcoma, a spontaneously derived tumor maintained in Sprague-Dawley rats, was examined for its morphological and biochemical characteristics. The tumor matrix exhibited cartilage-like-staining properties. Cartilage-specific type II collagen was identified intracellularly and following hyaluronidase pretreatment in the extracellular matrix by means of immunofluorescent techniques with antitype II antibodies. Histological studies revealed a multinodular tumor structure. Type I and III collagens were found predominantly in the septa and stroma between the nodules. Various procedures for obtaining the maximal yield of viable cells from the tumor were compared. Under the culture conditions used, primary cell suspensions in short-term culture did not change their phenotypic expression regarding the synthesis of type II collagen. Biologically active polysomes and messenger RNA were isolated from cell suspensions, which proved to be a better source for such macromolecules than was the total tissue. The Swarm rat chondrosarcoma is therefore a useful model system for studying the biosynthesis of type II collagen. A similar methodology could be used to study human tumors. The macromolecular synthetic pattern of malignant sarcomas, particularly the pattern of structural matrix proteins, may provide a more rational classification scheme for these poorly understood human tumors.

INTRODUCTION

The multiple types of collagen are now well established as different gene products that can be distinguished from one another chemically and chromatographically (15). A number of experimental solid tumors synthesize an extracellular matrix composed mainly of one type of collagen. This is in contrast to normal tissues which usually contain complex mixtures of collagens in their extracellular matrix (9).

A transplantable rat chondrosarcoma with a cartilaginous matrix has been demonstrated to synthesize primarily type II collagen. This collagen represents 50% of the total protein synthesized by the tumor (18). As might be expected, mRNA coding for type II procollagen is a major RNA species in this tumor (2).

In the present paper, we describe the morphological and histological features of the tumor. Collagen phenotypes were examined by immunofluorescence techniques using type I, II, and III collagen-specific antibodies. Biologically active mRNA and polysomes were best obtained from primary cell suspensions, rather than from the total tumor, tissue slices, or tissue homogenates.

We suggest that other soft tissue sarcomas might be studied in a similar fashion. Furthermore, the systematic characterization of human sarcomas, many of which have an unknown cell of origin, could be approached by using the techniques described herein.

MATERIALS AND METHODS

Tumor Growth. The chondrosarcoma tumor was maintained by serial transplantation in female Sprague-Dawley rats (6). Tumor cell suspensions in phosphate-buffered saline were injected subcutaneously into 3-week-old rats. The animals were fed ad libitum and sacrificed when the tumors had reached 15 to 20 g, usually 5 to 6 weeks after transplantation.

Histological Techniques. Tumor sections for light microscopy were fixed in phosphate-buffered formalin and stained with hematoxylin-eosin, Masson's trichrome, silver, alcian blue, or periodic acid-Schiff stains (1).

Immunological Procedures. Purified antibodies were prepared from rabbit antisera to bovine types I, II, and III collagens by immuneabsorption procedures as reported previously (20). Cryostat sections were prepared and processed for immunofluorescence according to the method of Gay et al. (8). Before they were reacted with anti-type II antibodies, some samples were treated with hyaluronidase.

Isolation of Collagen Synthesized in Vitro. Fresh tumor tissue was minced and treated with 0.1% collagenase and 0.05% hyaluronidase (Grand Island Biological Co., Grand Island, N. Y.), and the released cells were resuspended and washed in spinner medium (6). The cells were incubated with [3H]proline for 18 hr at 37° in Eagle's minimal essential medium containing 50 μg/ml-aminopropionitrile per ml. Collagen was isolated as previously described (18) and further purified by chromatography on DEAE-cellulose (14) and CM-cellulose (16).

Isolation of Polysomes. Total polysomes were isolated from tumor cells (5) by layering the 8000 x g supernatant of the cell lysate over cushions of 1 and 2 M sucrose and centrifuging at 105,000 x g for 2 hr at 2°.

Isolation and Separation of Labeled RNA. Tissue slices or cells were suspended in Eagle's spinner medium and incubated with [3H]uridine (Schwarz/Mann, Orangeburg, N. Y.) for 18 hr at 37°. RNA was extracted by the phenol procedure according to the method of Perry et al. (17) from either total tissue or postmitochondrial supernatants. Poly(A)2 RNA was isolated.

1 Supported in part by the Deutsche Forschungsgemeinschaft.
2 To whom requests for reprints should be addressed.

Received January 16, 1979; accepted September 17, 1979.
from total RNA by chromatography on poly(U)-Sepharose (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) and sedimented in denaturing sucrose gradients [5 to 20% sucrose-50% (v/v) formamide] as described elsewhere (3).

Hybridization with [3H]Poly(U). The presence of poly(A) sequences in RNA was confirmed by hybridization with [3H]-poly(U) according to the method of Gillespie et al. (10). After the RNA samples were incubated with [3H]poly(U) for 24 hr at 36°, they were incubated with pancreatic RNase for 2 hr at 30°. Radioactivity was determined following trichloroacetic acid precipitation at 0°.

RESULTS

The tumor was originally described as an osteogenic tumor that arose spontaneously in an adult female Sprague-Dawley rat (11). The initial tumor had chondroblastic components that resembled a cartilage neoplasm. After repeated transplantation of the tumor, only its chondrocytic elements persisted. At present, the tumor is clearly a chondrosarcoma with no other elements such as bone or osteoid present. The chondrosarcoma is routinely maintained by serial transplantation every 4 to 6 weeks, by which time it normally has reached an average weight of 15 to 20 g. No spontaneous regressions occurred, and no metastases were detected in the host during autopsy. The incidence of tumor formation following host inoculations is over 90%. (With the transplantation of several small pieces of tumor tissue s.c., the percentage of animals that developed a tumor was virtually 100%.)

Gross Morphology. The tumor was usually pink, translucent to opaque, grossly poorly vascularized, nodular, and with occasional cysts. The tissue was soft and not as firm as normal cartilage. Occasionally, a nidus of calcification was found, usually associated with hemorrhage and necrosis. (True osteoid was never observed microscopically.)

Histology. The tumor had an appearance typical of a malignant chondrosarcoma and was divided by septa into nodules. Within 1 to 2 weeks after transfer, the tumor grew to 2 to 4 mm in diameter. As the tumor enlarged, the traversing septa appeared to decrease relative to the bulk of the tumor. This is documented in Fig. 1, from tumor tissue examined at 2, 4, and 6 weeks after transplantation, respectively. When the tumors were allowed to grow for more than 6 to 7 weeks, the centers of nodules began to become necrotic, and there was an increased incidence of hemorrhage within the tumors.

The septa dividing the tumor contained cellular elements that differed markedly from those of the fibrous capsule. The latter contained typical fibroblast-like nuclei which could be observed after hematoxylin and eosin staining (Fig. 2A) but which were not apparent after staining with silver (Fig. 2B). Type I collagen fibrils are also apparent in Fig. 2B.

A gradient of cell size was present from the periphery of the nodule to the center (Figs. 3 and 4). Cells at the periphery of nodules tended to be smaller; the nuclei were more uniform, and the chromatin was more dense. At the center of nodules, more matrix was present, increasing the space between cells; nuclei were larger; and the Golgi apparatus was more pronounced. There was also a greater number of vacuoles in cells at the center of nodules than in cells at the periphery. Few mitotic figures were present within the nodules. It appeared that nodules increased in size by several mechanisms. Towards the center of a nodule, individual cells were larger, with a concomitant increase in matrix components. The nodules also appeared to grow by proliferation of cells, particularly at the periphery of the nodules. The vasculature of the tumor was generally restricted to the septal areas.

The matrix of this neoplasm stained in a manner similar to that of cartilage with alcian blue and periodic acid-Schiff stains. Although the extracellular matrix contained the highest concentrations of positively reacting substances, increasing amounts of staining was detectable intracellularly in cells at the center of nodules, suggesting an accumulation of secretory materials.

Elastin stains were particularly effective in revealing nuclear details in this tumor (Fig. 3). Cells with large, abnormal nuclei and multinucleated cells with marked nuclear atypia were evident.

Immunофluorescence. The nonspecificity associated with most staining procedures for light microscopy was partially overcome by using immunofluorescence techniques. We examined tumor slices with immunofluorescence stains using antibodies prepared against the various types of collagen. Frozen sections (6 μm) of the chondrosarcoma were treated with antibodies against type I, II, and III collagens. The chondrocytes and lacunar spaces showed fluorescence only with type II antibodies, confirming that these areas contained only cartilage-specific type II collagen (Fig. 5A). After the tumor slices were treated with hyaluronidase, fluorescence was also observed within the cartilaginous matrix in the extracellular areas (Fig. 5B). Apparently, the proteoglycans and glycosaminoglycans associated with collagen in the extracellular matrix prevented reaction with the specific antibodies unless a portion of the carbohydrate moieties was digested with the hyaluronidase (20). Positive staining for type I and III collagens was present only in the surrounding capsule and within the septa separating the tumor nodules (Fig. 5C and D, respectively).

Biochemical Analyses. Biochemical characterizations of the tumor were accomplished by using primary cell suspension under short-term culture conditions for analysis of macromolecular synthesis. In the course of these experiments, various procedures for tissue disruption were compared. The most important considerations were yield and viability of cells, as well as time needed for handling the tissue. Collagenase treatment combined with tissue disruption in a glass Dounce homogenizer improved the procedure considerably, and the addition of hyaluronidase further accelerated digestion. Since the material could be homogenized only after 60 to 90 min of pretreatment with enzyme, tissue disruption with sonication and homogenization (Polytron Apparatus; Brinkmann Instruments) before digestion was attempted. The tissue was dispensed directly in about two-thirds of final enzyme solution. Disruption was accomplished with the Polytron at the lowest possible setting for 20 to 30 sec. In most experiments, incubation for 2 hr at 37° in the presence of collagenase and hyaluronidase was sufficient to disintegrate the tissue completely into a suspension of single cells. When this procedure was used, cell damage was not apparent microscopically, and the yield of viable cells as determined by trypan blue exclusion was optimal.

An important criterion for the maintenance of in vivo function of isolated cells was to demonstrate the synthesis of type II...
collagen in suspensions of these cells. Cell suspensions were washed twice with medium containing 10% fetal bovine serum, washed once with incubation medium, and then incubated for 18 hr in the presence of [3H]proline. The incubation was terminated by chilling the cell suspensions on ice, and the labeled collagen was then extracted from the combined medium and cell fraction. After extraction, repeated salt precipitation, and dialysis of the labeled collagen, the sample was chromatographed on DEAE-cellulose to remove all the proteoglycans. It was further analyzed by chromatography on CM-cellulose in the presence of unlabeled type II collagen. As illustrated in Chart 1, approximately 90% of the eluted material comigrated with authentic unlabeled type II chains and was collagenase digestible. Very little radiolabeled material was found in the region of the α2 chains; the presence of such material would have indicated the presence of type I collagen.

The CM-cellulose chromatography suggests that type II collagen α chains are being synthesized by the tumor chondrocytes. Cartilage cells have been shown to “switch” from synthesis of [α1(II)]3 to [α1(I)]3 synthesis in vitro, albeit over a time span different from the one in these experiments (12, 13). Direct evidence that type II α chains were being made would come only from cyanogen bromide peptide mapping.

Polysomes were isolated for in vitro translation studies. Table 1 shows that polysomes obtained from cell suspensions were more active than those isolated from total tumor homogenates. The omission of either polysomes or energy source resulted in background levels of incorporation, as did pretreatment with RNase. As demonstrated elsewhere (6), 65% of the cell-free product is susceptible to collagenase digestion and is comparable in size to the type II collagen precursor pro-α chains. In addition, analysis of the size distribution of polysomes on linear sucrose gradients (Chart 2) revealed an improvement due mainly to the use of cell preparations. Chart 2, A and C, indicates the importance, as reported previously (6), of the proper ion concentration during the separation. As a control, polysomes were treated with EDTA, which destroyed all the higher-molecular-weight complexes (Chart 2, B and D). The major peaks represented small and large ribosomal subunits and monosomes. A common feature for most mammalian cytoplasmic mRNA’s is the presence of a poly(A)+ sequence at the 3'-end of the molecule. The presence of poly(A)+ can be detected by hybridization with [3H]poly(U). Alternatively, the poly(A)+ containing RNA can be isolated by chromatography on oligodeoxythymidylic acid cellulose or poly(U)-Sepharose. In Table 2, various RNA preparations are compared for poly(A) content. Although the values for hybridization with [3H]poly(U) found in cell extracts are similar to those found in other tissues, very little hybridizable material was detected in RNA extracted from whole-tumor tissue.

Newly synthesized poly(A)+ RNA was isolated from cell suspensions and separated on linear sucrose gradients under denaturing conditions. The labeled poly(A)+ RNA from cells showed a larger proportion of high-molecular-weight material (Chart 3B). In contrast, profiles of poly(A)+ RNA isolated from whole-tumor slices showed extensive degradation (Chart 3A). In addition, results obtained from cellular preparations tended to be more reproducible from one preparation to the next. On the basis of these results, we concluded that analysis of tumor

<table>
<thead>
<tr>
<th>Sample</th>
<th>[3H]Proline incorporation (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor cell polysomes and RNase A (80 μg/ml)</td>
<td>3,250</td>
</tr>
<tr>
<td>Tumor cell polysomes minus energy source (ATP, creatine phosphate, and creatine phosphokinase)</td>
<td>4,300</td>
</tr>
</tbody>
</table>

* The reaction mixture of 250 μl contained 0.5 A260 of polysomes.
cell suspensions in primary short-term culture was the most effective method for evaluating the biological properties and in particular the macromolecular synthesis in the chondrosarcoma.

**DISCUSSION**

The Swarm rat chondrosarcoma is derived from a tumor that originally arose spontaneously in a female Sprague-Dawley rat and has been maintained for several years by serial transfer. This tumor originally contained bone as well as cartilage elements. The tumor was originally termed as osteochondroma. However, the cartilaginous portion of the tumor appeared to show progressive growth on serial transplantation; with transplantation, the bone element disappeared. No further description of the tumor were made until 1971 (4) when it was described as a chondrosarcoma. This tumor has remained quite stable over the past 7 years. As shown in a representative area in Fig. 2, the entire tumor now consists of cartilaginous elements.

The marked stability of this tumor is in contrast with the observed behavior of many experimental tumors. The tumor is highly differentiated and grossly avascular and is composed almost exclusively of cartilage. One explanation for the stability of the phenotype may be that the tumor has been serially transplanted by R. Swarm who used small pieces of tumor tissue every 6 to 12 weeks. In contrast, many experimental tumors are transplanted at more frequent intervals. With the latter, it is possible that the most rapidly growing cells are constantly being selected. This may explain why many experimental tumors look remarkably similar and have the appearance of undifferentiated primitive mesenchymal cells.

Human soft tissue tumors have long presented a problem in diagnosis. These polymorphic tumors are usually diagnosed by using 1 of 2 classification schemes, that of Stout and Lattes (19) or that of the WHO (7). Each of these classification schemes has some disadvantages. In most cases in which the cell of origin of the tumor is not known, the diagnosis depends on a histological pattern. For example, terms such as synovial sarcoma, epithelioid sarcoma, alveolar soft part sarcoma, and malignant fibrous histiocytoma refer to the morphological features of tumors for which the cell of origin is unclear.

The nature of the structural matrix proteins that these tumors elaborate may help to develop a diagnostic scheme based on more defined biochemical criteria, rather than on only histological characteristics. The rat chondrosarcoma might represent an experimental model and prototype for comparison and analysis of human soft tissue tumors. Cells could be maintained in short-term primary cultures. With appropriate radiolabeled precursors, the components of the extracellular matrix which these tumor cells synthesize can be analyzed. In this way, it might be possible to establish on a biochemical basis the cells of origin for these tumors. Isolation of such biologically active cell components as polysomes and mRNA has great potential in this regard.

**REFERENCES**


Macromolecular Synthesis in a Chondrosarcoma


Fig. 1. Photomicrograph of sections of rat chondrosarcoma from a tumor removed 2 (A), 4 (B), and 6 (C) weeks, respectively, after the injection of 1 ml of tumor cell suspension. The nodules have increased in size with the progressive growth of the tumor, and septal areas occupy smaller portions of the total. Silver stain, × 25.
Fig. 2. A, photomicrograph of sections of rat chondrosarcoma, 6 weeks after the injection of 1 ml of tumor cell suspension. A portion of the capsule, which is dark and can be distinguished from the subcapsular tumor septum, is included. Silver stain, × 100. B, photomicrograph of a section of the same tumor in the region of the capsule. The cellular elements of the capsule contain fibroblast-like nuclei, which can be distinguished from subcapsular septal nuclei. H & E, × 250.

Fig. 3. Photomicrographs of the rat chondrosarcoma 6 weeks after the injection of 1 ml of tumor cell suspension, demonstrating the progression of cell maturation from the septum to the center of the tumor nodule. Silver stain, × 100 (A) and 250 (B).
Fig. 4. Photomicrograph demonstrating histological details of the rat chondrosarcoma 6 weeks after the injection of 1 ml of tumor cell suspension. Masson’s trichrome (A) and H & E, (B), × 250.
Fig. 5. Photomicrographs of frozen tissue sections of rat chondrosarcoma labeled with isolated rabbit antibodies against type II collagen and subsequently stained with fluorescein-labeled anti-rabbit globulin. In A, tissue not treated with hyaluronidase shows fluorescence on chondrocytes and lacunar surface only. In B, after treatment with hyaluronidase, the cartilagenous matrix is also labeled. C and D, capsule and connective tissue stroma of the chondrosarcoma stained with antibodies against type I (C) and type III (D) collagen. × 250.
Histological and Biochemical Studies of a Transplantable Rat Chondrosarcoma

Dirk Breitkreutz, Lino Diaz de Leon, Larry Paglia, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/39/12/5093

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.