Glycosaminoglycans in Transplantable Rat Malignant Fibrous Histiocytoma

Jyoji YAMATE, Masanori TAJIMA, Toshiki SAITOH, Masaharu TOGO, Kazumoto SHIBUYA, Miheko IHARA, and Satoru KUDOW

Nippon Institute for Biological Science, 2221-1 Shin-machi, Ome, Tokyo 198, Japan

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Malignant fibrous histiocytoma (MFH) is a soft-tissue tumor consisting of an admixture of fibroblastic cells and histiocytic cells. Human MFH has been classified into storiform, pleomorphic, myxoid, xanthogranulomatous and giant cell types on the basis of predominant cell types and the amount of intercellular material [6]. MFH has been induced by treating rats with various agents [9, 11], whereas spontaneous rat MFH is rare [8]. The precise histogenesis of MFH remains controversial, although undifferentiated mesenchymal cell, histiocyte or fibroblast has been proposed as an original cell type [8, 9, 11, 15].

We established a transplantable tumor (MFH-MT) from a spontaneous subcutaneous MFH arising in a 15-month-old male Fischer 344/DuCrj rat [19]. The tumors which developed in syngeneic rats or athymic nude mice by transplanting a piece of MFH-MT, and the tumors which were induced in syngeneic rats by inoculating *in vitro* passaged cells (MT-P) derived from MFH-MT showed highly variable morphologic patterns [19, 20]. Myxoid areas developed in MFH-MT were composed of loosely arranged spindle cells and polygonal cells as well as intercellular material [19, 20]. The intercellular material was positive for alcian blue (pH 2.5) and negative for periodic acid-Schiff, suggesting that the stroma of myxoid areas contains glycosaminoglycans (GAGs). GAGs consist of hyaluronic acid (HA), chondroitin sulfate A (ChS-A), chondroitin sulfate B (dermatan sulfate: DS), chondroitin sulfate C (ChS-C), keratan sulfate (KS) and heparin (HeP). The purpose of this study is to identify GAGs in MFH-MT by histochemical stainings and cellulose acetate electrophoresis (CAE).

The tumors examined histochemically were as follows: MFH-MT at passage 26 (MT-26) which had been serially passaged in syngeneic rats by subcutaneous transplantation; tumors induced

in the tail-root of syngeneic rats by intradermal transplantation of MFH-MT (MT-RT); tumors induced in subcutaneous tissue of CRJ: CD-1 (ICR)-nu/nu nude mice by subcutaneous transplantation of MFH-MT (MT-NU); and tumors induced in syngeneic rats by intraperitoneal inoculation with MT-P (MT-PT). Sections prepared from all these tumors contained myxoid areas. Enzymes used in histochemical examinations were bovine testicular hyaluronidase (BH) (Sigma Chemical Co., St. Louis), streptomyces hyaluronidase (SH) (Seikagaku Kogyo Co., Ltd.), chondroitinase ABC (C-ABC) (Sigma) and chondroitinase AC (C-AC) (Sigma). Specificity of these enzymes for GAG degradation was shown as follows: BH for HA, DS, ChS-A and ChS-C; SH for HA; C-ABC for HA, DS, ChS-A and ChS-C; C-AC for ChS-A and ChS-C [4]. BH was dissolved at 100 turbidity reducing units (TRU)/ml in 0.2 M acetate buffer, pH 5.6. Sections were incubated with the BH solution at 37°C for 3 hr. SH was dissolved at 50 TRU/ml in 0.1 M phosphate buffer, pH 6.0. Sections were incubated with the SH solution at 60°C for 4 hr. C-ABC and C-AC were dissolved in a concentration of 1-2 unit/ml in 0.1 M tris-HCl buffer, pH 8.1 and pH 7.4, respectively. Sections were incubated with the C-ABC solution or C-AC solution at 37°C for 2 hr. After being incubated with the enzymes, sections were stained with alcian blue and nuclear fast red. Control sections were processed in each of the buffers without the enzymes under the same conditions and stained.

Two samples of sticky cyst-fluid (C42-2 and C42-3) which were collected from passage 42 of MFH-MT in syngeneic rats, and culture medium which was collected 5 and 13 days after seeding passage 24 of MT-P (TC-5 and TC-13) were analyzed by CAE as will be described below. Culture medium incubated for 5 days in the absence of MT-P was analyzed in a like manner and served as the negative control (TC-C). Medium used for cell culture was Dulbecco's minimum essential medium (GIBCO) supplemented with 10% heat-inactivated fetal

Fig. 1. Part of myxoid area of MFH-MT developed at the tail-root of a Fischer 344 rat. The matrix stains positive with alcian blue. Alcian blue (pH 2.5) and nuclear fast red, x180.

Fig. 2. Part of myxoid area of the same MFH-MT as that shown in Fig. 1. Alcian blue-positive material has been completely digested by bovine hyaluronidase. Alcian blue (pH 2.5) and nuclear fast red after treatment with bovine hyaluronidase, x180.

bovine serum (GIBCO), streptomycin (100 μ g/ml) and penicillin (100 U/ml).

Extraction of GAGs and electrophoresis were performed according to the methods described by Hagari and Yumoto [10], with the following modifications: The extracted samples and standard GAGs including HA, DS, ChS-A, ChS-C, HeP and KS (Sigma) were dissolved in distilled water at concentrations of 0.5–1 mg/ml. For electrophoresis 2 μ l of these solutions was applied at 1 cm from the negative electrode on a 6 $\times$ 8 cm cellulose acetate sheet which had been presoaked in 0.1 M pyridine-formic acid buffer, pH 3.0. The sheets were subjected to electrophoresis for 10 min at 180 V in the same buffer and subsequently stained with alcian blue. The samples of C42-3, TC-13, standard HA and standard ChS-A were incubated twice in an SH solution (25 TRU/ml) for 24 hr at 50°C, and these samples were then subjected to CAE.

MT-26, MT-RT, MT-NU and MT-PT were similar histologically to each other in results of alcian blue stain combined with enzymatic degradation. GAGs in tumor tissues were completely digested by BH, moderately by C-ABC and C-AC, and faintly by SH, compared with controls positive for alcian blue stain (Figs. 1 and 2). These results demonstrated that the intercellular matrix of myxoid areas contained both HA and ChS. In CAE the bands of C42-2, C42-3, TC-5 and TC-13 appeared to correspond to those of the standard HA, but did not correspond to those of the other standard GAGs (Figs. 3 and 4). The band of TC-C did not appear

Fig. 3. Cellulose acetate electrophoresis of glycosaminoglycans in cyst-fluid. S, start; 1 (C42-3) and 2 (C42-2), samples of cyst-fluid from MFH-MT; 3, standard heparin (HeP); 4, standard chondroitin sulfate C (ChS-C); 5, standard chondroitin sulfate B (dermatan sulfate: DS); 6, standard chondroitin sulfate A (ChS-A); 7, standard keratan sulfate (KS); 8, standard hyaluronic acid (HA). The bands of lanes 1 and 2 appear to correspond to that of lane 8.

on the sheet (Fig. 4). C42-3, TC-13 and standard HA were degraded when treated with SH solution which digests only HA (Fig. 5). However,

Fig. 4. Cellulose acetate electrophoresis of glycosaminoglycans in culture medium. S, start; 1, culture medium harvested 5 days after seeding of MT-P (TC-5); 2, culture medium harvested 13 days after seeding of MT-P (TC-13); 3, culture medium incubated for 5 days in the absence of MT-P (TC-C); 4, Hep; 5, DS; 6, ChS-A; 7, KS; 8, HA. The bands (arrow) of lanes 1 and 2 apparently correspond to that of lane 8, whereas the corresponding band is absent in lane 3. See abbreviations in Fig. 3.

Fig. 5. Cellulose acetate electrophoresis of glycosaminoglycans treated with streptomyces hyaluronidase (SH). S, start; 1, ChS-A+SH; 2, HA+SH; 3, TC-13+SH; 4, C42-3+SH; 5, ChS-A; 6, HA; 7, TC-13; 8, C42-3. Although part of the band of lane 4, which slightly migrates toward the positive electrode, faintly appears, bands of lanes 2, 3 and 4 are missing by SH treatment in contrast to those of no treated samples in lanes 6, 7 and 8, respectively. The band of lane 1 appears similar to that of lane 5, indicating that ChS-A is not digested by SH. See abbreviations in Figs. 3 and 4.

part of the band of C42-3 which slightly migrated toward the positive electrode was not degraded, probably due to the difference in molecular size. The results of CAE suggested that cells constituting MFH-MT may secrete HA.

The presence of HA has histologically been demonstrated in human MFH [15] and in MFH of mice induced by intraperitoneal inoculation of peritoneal macrophages transformed by simian virus 40 [10]. In these mice HA was also identified in their ascites by CAE [10]. There have been no reports on GAGs in rat MFH.

HA, which was synthesized at the inner side of plasma membranes [14], has been known to play an important role in the development of embryonal and primitive cells [12, 16]. HA has also been shown to be present in a variety of neoplastic tissues [4, 5]. Particularly, mesothelioma was reported to secrete a considerable amount of HA, that was regarded as one of the diagnostic criteria for this tumor [5, 18]. Ex-

perimentally induced mesotheliomas in rats were considered to be derived from a multipotential cell capable of differentiating into both fibroblasts and epithelial cells [3]. Neoplastic cells forming MFH-MT might be at an embryonal and undifferentiated stage, because they produce HA as mentioned by Roholl *et al.* [15]. Mammary tumors with cartilagenous tissue and osteoids of dogs contained both HA and ChS in their extracellular matrix [13]. Both GAGs demonstrated in MFH-MT by our histochemical examinations may be related to osteogenesis, since an osteosarcoma-like structure consisting of osteoblasts, osteoids and calcifying tissue was sporadically found in MFH-MT [19, 20].

Recently, GAGs have been studied in association with tumor malignancy such as invasiveness, adhesiveness and metastasis [1, 2, 4, 17]. Exogenous cell growth factor was shown to exist in GAGs produced by bone marrow stromal cells [7]. These observations may explain the rela-

tionship between cell growth and GAGs. The significance of GAGs in rat MFH remains to be elucidated by further studies.

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要 約

ラットの可移植性悪性線維性組織球腫 (MFH-MT) におけるグリコサミノグリカン (GAG) (短報): 山手丈至・田島正典・斎藤敏樹・東郷正治・渋谷一元・伊原三重子・工藤 悟 (日本生物科学研究所) ——MFH-MT の粘液領域に存在する GAG を組織化学的に証明した。GAG は、ウシ精巣ヒアルロニダーゼで完全に消化され、次いでコンドロイチナーゼ ABC 及びコンドロイチナーゼ AC で中等度に、streptomyces 産生ヒアルロニダーゼで軽度に消化された。粘液領域にはコンドロイチン硫酸とヒアルロン酸 (HA) が存在することが示された。MFH-MT より採取したシスト液及び MFH-MT に由来する培養細胞の培養上清から GAG を抽出し、標準 GAG と共にセルロースアセテート膜上で電気泳動を行った。その結果、HA が検出され、MFH-MT 構成細胞は HA を分泌することが証明された。