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# Hyaluronic acid in a cardiac myxoma: a biochemical and histological analysis

**Michael T. Longaker[1], Ernest S. Chiu[2], Benjamin Hendin[2], Walter E. Finkbeiner[2], and Robert Stern[2]**

Departments of [1] Surgery and [2] Pathology, School of Medicine, University of California, San Francisco, CA 94143, USA

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**Summary.** Cardiac myxoma is the most common primary tumor of the heart. This tumor has a gelatinous stroma that is thought to be composed of glycosaminoglycans, the classical acid mucopolysaccharide ground substance. We examined both biochemically and histologically the hyaluronic acid in a case of cardiac myxoma using a newly developed hyaluronic acid-binding protein probe. We observed that hyaluronic acid was localized in the amorphous stroma and occurred at levels 30 times that found in normal atrial septum.

**Key words:** Cardiac myxoma – Hyaluronic acid – Extracellular matrix

## Introduction

Cardiac myxomas are the most common primary tumors of the heart (McAllister and Fenoglio 1978). These tumors contain a variety of cell types that are thought to arise from a foci of primitive pluripotential mesenchymal cells in the area of the fossa ovalis (Wold and Lie 1980). These undifferentiated cells may subsequently undergo transformation and proliferation leading to tumor formation. However, the bulk of these tumors is composed of loose hydrated extracellular matrix described by classical pathologists as an acid mucopolysaccharide-rich "ground substance." This gelatinous substance in cardiac myxomas must often be "scooped out" during surgical excision of the tumor. We postulated that the jelly-like stroma was, in part, hyaluronic acid (HA). HA is a glycosaminoglycan that is prominent during tissue remodelling, rapid regeneration and repair, embryogenesis, and carcinogenesis. HA is particularly prominent in the developing heart (Orkin and Toole 1978; Markwald et al. 1978). We have previously demonstrated histochemically that HA is contained in the gelatinous stroma of cardiac myxomas (Hendin et al. 1991). In the present study we provide coordinated biochemical quantitation and histolocalization, using a newly developed probe to demonstrate that HA is prominent in the stroma in this case of a cardiac myxoma.

*Offprint requests to:* R. Stern

## Case report

A 57-year-old man presented to the Cardiothoracic Surgery Service at the Medical Center of the University of California, San Francisco, with a 2-month history of easy fatigability, shortness of breath, and paroxysmal nocturnal dyspnea. His past medical history was remarkable for a myocardial infarction at age 39. Physical examination revealed a 3/6 diastolic murmur. Cardiac catheterization demonstrated a large mass in the left atrium and high-grade stenosis of the right and left circumflex coronary arteries. During cardiopulmonary bypass, a large encapsulated mass arising from the anterolateral commissure of the mitral valve was found filling the entire left atrium. The tumor was excised together with a rim of mitral valve. The valve was replaced with a prosthetic valve and right and circumflex arteries were bypassed using saphenous vein grafts. His postoperative course was unremarkable. A representative portion of the fresh surgical specimen was frozen and the remainder fixed in formalin.

The surgical specimen was an encapsulated, nearly spherical mass of $5.3 \times 4.5 \times 3.2$ cm, and weighted 42.75 g. The bisected specimen was brown and had a shiny gelatinous texture. Hematoxylin-and-eosin-stained sections of the mass revealed a typical myxoid stroma of diffuse ground substance. Polygonal cells within the stroma had a weakly eosinophilic cytoplasm.

For comparison, a section of atrial septum from a normal heart was obtained at autopsy from a 51-year-old man who died of a metastatic tumor. A portion was immediately frozen and the remainder fixed in formalin.

### Methods

*Preparation of atrial myxoma for HA content analysis.* The gelatinous tumor was minced with scalpels. The tumor was homogenized for 30 s in a Biospec blender (Bartlesville, Okla.) while immersed in an ice bath. The methods of Scott (1960) and of Laurent and Tengblad (1980), with some modification, were employed for HA extraction. Briefly, 1-ml samples of tumor were boiled at 100° C for 15 min, placed on ice, and digested with 250 µl of 2× crystal-

lized, preactivated papain (19 units/mg, Sigma, St. Louis, Mo.) at 70° C for 18 h. Papain digests were spun at 11000 *g* in a Beckman 11 centrifuge. A 500-µl aliquot was passed over a 1.0 × 18 cm column of Sephadex G-25 (Pharmacia, Uppsala, Sweden) equilibrated with the assay buffer (1.5 NaCl, 25 mM phosphate buffer, pH 7.0, containing 0.3 M guanidine HCl, 0.08% bovine serum albumin, and 0.02% $NaN_3$).

*Preparation of HA Sepharose gel and HA binding protein.* Sepharose (AH Sepharose-4B, Pharmacia, Uppsala, Sweden) was covalently linked to HA (ICN, Lisle, Ill.) using the carbodiimide procedure as described by Tengblad (1979). Hyaluronic acid binding protein (HABP) was extracted from bovine nasal cartilage (Pelfreeze, Rogers, AR) as described by Hendin et al. (1991).

*[$^{35}$S]Streptavidin HABP assay.* All samples were assayed using a newly developed [$^{35}$S] streptavidin HAPB assay modified from Tenglbad (1979). Briefly, 100-µl samples of equilibrated digests were aliquoted into the upper chambers of CoStar Spin-X tubes (CoStar, Cambridge, Mass.) and 150 µl of HABP diluted 1:50 in assay buffer was then added. The solutions were mixed and rotated on an American Rotator V (American Scientific, McGaw Park, Ill.) for 30 min at 160 rpm at room temperature to allow the HA in the tissue extract to bind to HABP. Next, 60 µl of HA gel diluted 1:5 as above was added. The solutions were mixed and rotated as above for 60 min to allow the free HABP to bind to the gel. As samples were spun at 11000 *g* for 5 min, the filtrate passed into the filter unit's lower chamber. The HA gel was resuspended in 200 µl of assay buffer to wash and then spun for 5 min as above. Next, 200 µl of [$^{35}$S] streptavidin (1000 Ci/mmol, 100 µCi/ml; Amersham, UK) diluted 1:200 in PBS-CMP (phosphate-buffered saline; calcium-magnesium-free) was added and the pellet was again resuspended. Samples were rotated for 30 min, then spun for 5 min as above. As a final wash, the pellet was resuspended in 200 µl PBS-CMF and spun as above. The HA-gel pellet was transferred to counting vials with distilled water and Optifluor scintillation fluid (Packard, Downers Grove, Ill.) was added. Non-specific streptavidin binding was determined by assayed samples without binding protein. This was subtracted from all values. A standard curve was constructed using known concentrations of HA. Control samples without HA were equal to 100% bound. All samples were assayed in triplicate. The data for each sample, run in triplicate, were expressed as the mean value ± standard deviation.

## Results

Portions of normal cardiac tissue, both fresh and fixed in formalin, were obtained for comparison to fresh tissue from a surgically excised myxoma. The biochemical determinations using the [$^{35}$S] assay are shown in Table 1. The highest concentration of HA, as expected, was found in the cardiac myxoma. The calculated HA concentration in the myxoma was 7.84 ± 1.68 µg/mg wet weight. This level was more than 30 times the level of HA found in normal atrial septum (0.25 ± 0.05 µg/mg wet weight). Interestingly, the level of HA was almost identical in non-fixed and formalin-fixed cardiac tissue.

**Table 1.** Hyaluronic acid analysis

| Sample | Hyaluronic acid (µg/g wet weight) |
|---|---|
| 1. Myxoma | 7.84 ± 0.27 |
| 2. Normal heart (fresh) | 0.25 ± 0.06 |
| 3. Normal heart (formalin fixed) | 0.26 ± 0.05 |

In morphologic studies, the myxoma was stained with the same biotinylated HABP utilized in the biochemical determinations employing an avidin-biotin-complex (ABC) procedure. The tumor stroma stained positively using the HABP. The staining was markedly reduced when the probe was pretreated with HA to saturate the binding sites. Staining with streptavidin-horseradish peroxidase and CMF-PBS without the addition of HABP was used as an additional control (data not shown).

## Discussion

The rapid development of echocardiography and magnetic resonance imaging has improved our ability to diagnose cardiac myxomas (Roudaut et al. 1987; Talley and Wenger 1987; Freedberg et al. 1988). These tumors are the most common primary tumors of the heart, constituting between 25% and 50% of all heart tumors (McAllister and Finoglio 1978). There are several theories as to the origin of these tumors: they are thought to arise from (1) a rest of primitive cardiac cells; (2) pleuripotential subendocardial cells; or (3) from fetal cells about the fossa ovalis that are maintained in a dedifferentiated state, which then undergo transformation and proliferation (McAllister and Finoglio 1978; Morales et al. 1980; Wold and Lie 1980; Govoni et al. 1988).

It was suspected that HA was present in cardiac myxomas from several prospectives: their soft gelatinous nature, the previous demonstration of high levels of HA in the cardiovascular system (Markwald et al. 1978; Orkin and Toole 1978; DeReeder et al. 1988; Hendin et al. 1990); and a stroma that stains positively for alcian blue, suggesting a composition rich in glycosaminoglycans. Using a newly developed biotinylated HABP assay coupled to [$^{35}$S] streptavidin, we demonstrated that HA was present in the stroma of a cardiac myxoma. We found 7.84 ± 1.68 µg/mg wet weight HA after extraction, protease digestion, and Sephadex G-25 chromatography. The papain digestion and Sephadex G-25 chromatography were modifications of previous techniques (Scott 1960; Laurent and Tengblad 1980) and were performed to remove proteins, lipids, and other substances that bind to HA and could potentially interfere with the [$^{35}$S]HABP-based assay. Substantial levels of HA were present in the myxoma, over 30 times that seen in normal atrial septum. Actual levels are likely to be even higher, as not all HA (particularly protein-bound HA) was recovered by the extraction technique.

We have recently described a glycoprotein in fetal serum that stimulates the production of HA (Decker et al. 1989). Very little information is available about the mechanisms involved in controlling HA deposition. This glycoprotein factor may modulate HA deposition during fetal development, oncogenesis, and tissue repair (Decker et al. 1989; Longaker et al. 1989). In addition, we have observed high levels of this activity in extracts of the fetal heart, second only to kidney among all fetal organs (Longaker et al. 1990). This observation was an

additional reason for suspecting a prominent role of HA in cardiac myxomas. This also suggests pathophysiologic mechanisms for the development of cardiac myxomas, a hypothesis that is now being pursued.

The histochemical studies support our biochemical results that demonstrated HA in increased levels in cardiac myxomas. The negative control for our morphologic studies was preincubation of the HABP with HA to saturate all potential binding sites. We observed that incubation of tissue sections with hyaluronidase, in contrast, did not remove all HA-positive staining in tissue sections. This is consistent with other observations from this and other laboratories that HA-protein complexes in tissue sections protect HA from complete hyaluronidase digestion (Burd et al. 1989). We have observed that in order for hyaluronidase to digest HA in its entirety, tissue sections must undergo preliminary digestion with papain at 70° C, a procedure which destroys tissue morphology. We conclude that the use of hyaluronidase predigestion as a routine control for HA staining in histopathology must be re-evaluated.

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## References

Burd DAR, Siebert JW, Ehrlich HP, Garg HG (1989) Human skin and post-burn scar hyaluronan: demonstration of the association with collagen and other proteins. Matrix 9:322–327

Decker M, Chiu ES, Dollbaum C, Moiin A, Hall J, Spendlove R, Longaker MT, Stern R (1989) A hyaluronic acid-stimulating activity in sera from the bovine fetus and from breast cancer patients. Cancer Res 49:3499–3505

DeReeder EG, Girard N, Poelmann RE, Van Munstreren JC, Patterson DF, Gittenberger-De Groot AC (1988) Hyaluronic acid accumulation and endothelial cell detachment in intimal thickening of the vessel wall; the normal and genetically defective ductus arteriosus. Am J Pathol 132:574–585

Freedberg RS, Kronzon I, Rumancik WM, Liebeskind D (1988) The contribution of magnetic resonance imaging to the evaluation of intracardiac tumors diagnosed by echocardiography. Circulation 77:96–103

Govoni E, Severi B, Cenacchi G, Laschi R, Pileri S, Rivano MT, Alampi G, Branzi A (1988) Ultrastructural and immunohistochemical contribution to the histogenesis of human cardiac myxoma. Ultrastruct Pathol 12:221–233

Hendin BN, Longaker MT, Finkbeiner WE, Roberts LJ, Stern R (1991) Hyaluronic acid deposition in cardiac myxomas: localization using a hyaluronate-specific binding protein. Am J Cardiovasc Pathol (in press)

Laurent UBG, Tenglbad A (1980) Determination of hyaluronate in biological samples by a specific radio assay technique. Anal Biochem 109:382–394

Longaker MT, Harrison MR, Crombleholme TM, Langer JC, Decker M, Verrier ED, Spendlove R, Stern R (1989) Studies in fetal wound healing. I. A factor in fetal serum that stimulates deposition of hyaluronic acid. J Pediatr Surg 24:789–792

Longaker MT, Adzick NS, Sadigh D, Hendin B, Stair SE, Harrison MR, Spendlove R, Stern R (1990) Hyaluronic acid-stimulating activity in the pathophysiology of Wilms' tumors. J Natl Cancer Inst 82:135–139

Markwald RR, Fitzharris TP, Bank H, Bernanke DH (1978) Structural analysis on the matrical organization of glycosaminoglycans in developing endocardial cushions. Dev Biol 62:292–316

McAllister HA, Fenoglio JJ (1978) Tumors of the cardiovascular system. Atlas of tumor pathology. Armed Forces Institute of Pathology, Washington, D.C., pp 5–20

Morales AR, Fine G, Castro A, Nadji M (1980) Cardiac myxoma (endocardioma): an immunocytochemical assessment of histogenesis. Hum Pathol 12:896–899

Orkin RW, Toole BP (1978) Hyaluronidase activity and hyaluronate content of the developing chick embryo heart. Dev Biol 66:308–320

Roudaut R, Gosse P, Dallocchio M (1987) Rapid growth of a left atrial myxoma shown by echocardiography. Br Heart J 58:413–416

Scott JE (1960) Aliphatic ammonium salts in the assay of acidic polysaccharides from tissue. Methods Biochem Anal 8:145–197

Talley DJ, Wenger NK (1987) Atrial myxoma: overview, recognition, and management. Comp Ther 13:12–18

Tengblad A (1979) Affinity chromatography on immobilized hyaluronate and its application to the isolation of hyaluronate binding proteins from cartilage. Biochim Biophys Acta 578:281–289

Wold LE, Lie JT (1980) Cardiac myxomas: a clinicopathologic profile. Am J Pathol 101:219–233