Short Communication

Cardiomyogenic Differentiation in Cardiac Myxoma Expressing Lineage-Specific Transcription Factors

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We investigated five cases of cardiac myxoma and one case of cardiac undifferentiated sarcoma by light and electron microscopy, *in situ* hybridization, immunohistochemical staining, and reverse transcriptase-polymerase chain reaction for cardiomyocyte-specific transcription factors, Nkx2.5/Csx, GATA-4, MEF2, and eHAND. Conventional light microscopy revealed that cardiac myxoma and sarcoma cells presented variable cellular arrangements and different histological characteristics. Ultrastructurally, some of the myxoma cells exhibited endothelium-like or immature mesenchymal cell differentiation. Immunohistochemistry for Nkx2.5/Csx, GATA-4, and eHAND was slightly to intensely positive in all myxoma cases. MEF2 immunoreactivity was observed in all cases including the case of sarcoma, thus suggesting myogenic differentiation of myxoma or sarcoma cells. *In situ* hybridization for Nkx2.5/Csx also revealed that all myxoma cells, but not sarcoma cells, expressed mRNA of the cardiac homeobox gene, Nkx2.5/Csx. Furthermore, nested reverse transcriptase-polymerase chain reaction from formalin-fixed, paraffin-embedded tissue was performed and demonstrated that the Nkx2.5/Csx and eHAND gene product to be detected in all cases, and in three of six cases, respectively. In conclusion, cardiac myxoma cells were found to express various amounts of cardiomyocyte-specific transcription factor gene products at the mRNA and protein levels, thus suggesting cardiomyogenic differentiation. These results support the concept that cardiac myxoma might arise from mesenchymal cardiomyocyte progenitor cells. (Am J Pathol 2002, 161:381–389)

These tumors continue to generate interest because of their unique location, varying clinical presentation, and uncertain histogenesis. Previous studies to identify the cells of origin of cardiac myxomas using immunohistochemistry, electron microscopy, and tissue culture have yielded conflicting results.^{3–7} Differentiation toward endothelial,^{3–6} fibroblastic,⁵ and smooth muscle cells,^{3,5} along with typical stromal cells, has been identified in cardiac myxoma, and glandular differentiation has rarely been observed.^{5,7} Neurogenic differentiation has also been suggested based on the findings of S100 protein and neuron-specific enolase immunoreactivity in some tumor cells.^{5,7} It is now believed that they may develop from primitive, reserve multipotential mesenchymal cells that are capable of several types of differentiation.^{5–7}

A recent genetic analysis of the embryonic patterning in the fruit fly led to the identification of the homeobox or *Hox* genes,⁸ which are encoding transcription factors, that appear to specify the formation of segmental structure along the A/P axis of embryos. Nkx2.5/Csx, is one of the mammalian homologues of *tinman*, a *Drosophila* homeobox gene required for the specification of cardiac precursor cells and for morphogenesis of the heart.^{9,10} Nkx2.5/Csx is first expressed in the presumptive precardiac mesoderm before gastrulation, but is later restricted to the bilateral dorsal regions that will develop into the muscular portions of the heart and is maintained throughout development.^{9,11} The expression of Nkx2.5/Csx during cardiomyogenesis is required for cardiac septation, in which a single atrium and ventricle are separated into four chambers.^{11,12}

At least four families of transcription factors have been reported to be expressed in the cardiac primordia fated to form the heart: NK homeodomain proteins,^{8–11} the myocyte enhancer binding factor-2 (MEF2),¹³ the zinc finger containing-GATA factors,^{14,15} and the basic helix-loop-helix proteins, dHAND and eHAND.¹⁶ These transcription factors appear to be expressed earlier in the

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Primary tumors of the heart are quite rare; however, among them cardiac myxomas are the most common ones.^{1–3}

Table 1. Clinical Data for Five Cases of Cardiac Myxoma and One Case of Cardiac Sarcoma

Case	Sex	Age	Tumor location	Tumor size (cm)	Weight (g)	Histological diagnosis	Examined methods*
1	F	69	Left atrial septum	6.7 × 5.0 × 2.6	50	Myxoma	IHC, ISH, TEM, RT-PCR
2	F	64	Left atrial appendage	6.3 × 5.0 × 4.0	70	Undifferentiated sarcoma	IHC, ISH, TEM, Nested RT-PCR
3	F	67	Left atrial septum	2.1 × 1.7 × 2.6	6	Myxoma	IHC, ISH, Nested RT-PCR
4	M	50	Left atrial septum	3.5 × 2.8 × 3.3	19	Myxoma	IHC, ISH, Nested RT-PCR
5	F	59	Left atrial septum	5.8 × 5.0 × 2.4	39	Myxoma	IHC, ISH, Nested RT-PCR
6	F	67	Left atrial septum	6.5 × 4.5 × 3.4	61	Myxoma	IHC, ISH, Nested RT-PCR

*IHC, Immunohistochemistry; ISH, *in situ* hybridization; TEM, transmission electron microscopy; nested RT-PCR, nested reverse transcriptase-polymerase chain reaction.

cardiogenic region during heart development, whereas in the postnatal heart, the expressions are highly restricted to the cardiac muscle cells. They are known to cooperatively interact with other regulatory factors to effect cardiac muscle differentiation.^{11,12}

Of particular interest is the demonstration of the expression of cardiomyocyte-specific transcription factors in clinical specimens that may molecularly define its cardiomyogenic differentiation, thus suggesting this to be an effective method for gaining insight into the histogenesis of this previously unclassifiable neoplasm, namely cardiac myxoma.

Materials and Methods

Patients and Tissue Samples

We examined cardiac tumor specimens from five patients with histologically diagnosed cardiac myxoma and one patient with cardiac undifferentiated sarcoma, and 11 tumor tissue specimens from other sites. All patients had undergone surgery at Saiseikai Central Hospital from 1994 to 2001 (Tables 1 and 4). Parts of the tumors were fixed in 10% neutralized formalin and embedded in paraffin. Sections measuring 2 to 3 μ m in thickness were subjected to conventional light microscopy, immunohistochemistry, and *in situ* hybridization. Tissue specimens obtained from a recent case (case 1) were immediately frozen in liquid nitrogen and kept at -80°C until use.

Antibodies

Goat anti-Nkx2.5/Csx, anti-eHAND and anti-GATA-4, and rabbit anti-MEF2 polyclonal antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Normal goat serum was from DAKO (Glostrup, Denmark).

Immunohistochemical Studies

Deparaffinized sections were examined as previously described.¹⁷ Briefly, the sections were incubated with 0.3% H_2O_2 for 30 minutes to remove any endogenous peroxidase activity and then were rinsed with Tris-buffered

saline. Antigenic epitopes were unmasked by an autoclave pretreatment. Primary antibodies (1:100 dilution) were added to the sections and incubated at 4°C overnight. After each section was incubated with biotin-free dextran (EnVision, DAKO) or (Histofine, Nichirei, Japan), the bound antibody was visualized with 3,3'-diaminobenzidine tetrahydrochloride, and the cell nuclei were counterstained with hematoxylin for 1 minute.

In Situ Hybridization

A Nkx2.5/Csx-anti-sense oligonucleotide probe (5'-GCT GCT GCT CCA GGT TTA GGA TGT CTT TGA CTG-3'), the corresponding sense probe (5'-CAG TCA AAG ACA TCC TAA ACC TGG AAC AGC AGC AGC-3'), and the eHAND anti-sense probe (5'-GAG AAA GAG CCA GAT AGG GAA ATG GAG ATA GGG CTG-3') were synthesized and labeled with biotin at the 5' ends. A sense probe was used as a negative control. Hybridization was performed on formalin-fixed paraffin sections using the GenPoint System (DAKO), according to the manufacturer's protocol. After performing the antigen retrieval method, the sections were incubated with a biotin-conjugated probe (1 $\mu\text{g}/\text{ml}$) at 37°C overnight. Each section was sequentially incubated with horseradish peroxidase-conjugated streptavidin, biotinylated tyramide, and streptavidin-biotin-peroxidase complex, and then was developed by 3,3'-diaminobenzidine tetrahydrochloride.

Transmission Electron Microscopy

The cardiac myxoma and sarcoma tissue specimens from cases 1 and 2 were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in a graded alcohol series, embedded in epoxy resin, and cut on an LKB ultramicrotome. Ultrathin sections on 150-mesh grids were then double stained with uranyl acetate and lead citrate and viewed under a JEOL-1200EXII electron microscope.¹⁷

RNA Extraction from Paraffin Sections

The cardiac myxoma and sarcoma samples had been fixed in 10% formalin, embedded in paraffin, and stored

Table 2. RT-PCR Primer Sequences and Product Sizes

	Primer sequence	Product size
Nkx2.5/Csx	5'-TATAACGCCTACCCCGCCTAT-3' 5'-TAATCGCCGCCACAACTCTCC-3'	420 bp
MEF2C	5'-TAATGGGGAAGGGGGTTCGATA-3' 5'-CTTCATGGCTTAGGGTAGTGCT-3'	810 bp
eHAND	5'-GCATGGAGCGCGCTTAAT-3' 5'-GGGCCTTTCCTGATGACA-3'	174 bp
GATA-4	5'-TCCTCTGCCTGGTAATGACTCC-3' 5'-CTCAGATCCTTCGGTGCTAGA-3'	260 bp
MLC-2v	5'-TCGAACAGACCCAAATCCAGG-3' 5'-TCCTAGTCCTTCTCTTCCG-3'	444 bp
β -actin	5'-ATCTGGCACCACACCTTCTACAATGAGCTGCG-3' 5'-CGTCATACTCCTGCTTGCTGATCACAATCTGC-3'	838 bp
Nkx2.5/Csx (first round)	5'-TATAACGCCTACCCCGCCTAT-3' 5'-TAATCGCCGCCACAACTCTCC-3'	420 bp
Nkx2.5/Csx (second round)	5'-CCGCCGCCAACAACAACCTC-3' 5'-GCTGTTGAGGTGGGATCGGT-3'	168 bp
eHAND (first round)	5'-TTCAGCCTTGCCCGACTCT-3' 5'-TTGGGTCCTGAGCCTTCCG-3'	439 bp
eHAND (second round)	5'-GCATGGAGCGCGCTTAAT-3' 5'-GGGCCTTTCCTGATGACA-3'	174 bp

at room temperature for 0.2 to 5 years. Total RNA was extracted from the paraffin sections as previously described.^{18,19} Briefly, the tissue sections were deparaffinized. Using the serial histological features of the hematoxylin and eosin-stained sections as a guide, the tumor cells were scraped off with a knife and collected in a tube, and then were incubated at 56°C for 48 hours in 200 μ l of digestion buffer containing 20 mmol/L Tris (pH 8.0), 20 mmol/L ethylenediaminetetraacetic acid, 2% sodium dodecyl sulfate, and 400 μ g/ml proteinase K. Total RNA was extracted using Trizol reagent (Life Technologies, Inc., Gaithersburg, MD), and then were resuspended in 10 to 30 μ l of RNase-free water. The RNA solution was treated with DNase I (Life Technologies, Inc.) for 15 minutes at 37°C.²⁰

Nested Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Complementary DNA was synthesized using 2 μ g of dissolved RNA, 1 μ l of random primer (Life Technologies, Inc.), and 200 U of reverse transcriptase (Superscript II, Life Technologies, Inc.). All of the primer sequences used in the present study are listed in Table 2. The first round RT-PCR products diluted with water to 1:50, including those of negative or positive controls, were used in the second round PCR.¹⁹ The PCR products were detected by ethidium bromide staining on 3.5% agarose gel.

Results

Conventional Light Microscopic Observations

Of all of the examined cases (cases 1 to 6), all of the tumor tissue revealed the abundant peculiar myxoid matrix and the tumor histology from cases 1 and 4 to 6 was the conventional type of cardiac myxoma. The sections demonstrated a paucicellular tumor characterized by

abundant, and sparsely cellular myxomatous but partly collagenized stroma and intervening satellite and spindle-shaped tumor cells appearing singly, in cords, and surrounding small blood vessels. Spindle or round tumor cells aggregated in the strands or irregular tubular structures (Figure 1A) with scattered mononuclear inflammatory cell infiltration. Hemosiderin depositions were frequently observed in the tumor tissue.

The myxoma tissue from case 3 histologically demonstrated massive bone structures and tubule formations by epithelioid tumor cells. The tumor had a hard calcified area with massive fibrosis, calcification, and ossification. Case 2 was an undifferentiated type of cardiac sarcoma containing rather primitive tumor cells with high cellularity, nuclear atypism, and hyperchromatism. Although in the small area, the sarcoma cells formed minute ossicles, undifferentiated primitive malignant cells with focal abundant myxoid matrix were the dominant constituents of the tumor tissue.

The findings of a histopathological diagnosis of the other 11 examined tumor tissue specimens were described in Table 4.

Light Microscopic Immunohistochemistry

The immunohistochemical results are summarized in Table 3 and are shown in Figure 1, B to I. Positive immunostaining for MEF2 was obtained in all cardiac tumor cases, including the case of cardiac sarcoma (Figure 1, B and D). In all cases, the tumor cells showed slightly to moderately positive staining in intensity. Positive staining was observed and condensed in the nucleus of the myxoma cells (Figure 1B). Nontumoral cardiomyocytes in the sections also showed intensely positive staining in the nucleus and worked as positive internal controls in the examined sections (Figure 1C). Mononuclear inflammatory cells in the sections showed no staining and worked as negative internal controls. The cardiac sarcoma cells showed a focal and

Table 3. Summary of Immunohistochemical and *In Situ* Hybridization Findings

Case	Histology	Immunohistochemistry				<i>In situ</i> hybridization	
		Nkx2.5/Csx	eHAND	GATA-4	MEF2	Nkx2.5/Csx	eHAND
1	Myxoma	+/-	+	+	+	+	+
2	Sarcoma	-	-	-	+	-	ND [‡]
3	Myxoma	+/-	+	++	+	+	ND [‡]
4	Myxoma	+	+	+	+	+	ND [‡]
5	Myxoma	+	++	+	+	+	ND [‡]
6	Myxoma	+	+/-	++	+/-	+	ND [‡]
1, 2, 4, 6*	Nontumoral cardiac muscle	+/-	+/-	+	++	+/-	+ [†] (only case 1)
Summary of positive stained structures		Nucleus and cytoplasm	Nucleus and cytoplasm	Nucleus	Nucleus	Nucleus and cytoplasm	Nucleus

++, Intensely positive; +, moderately positive; +/-, slightly and/or focally positive; -, negative.

*Immunohistochemically stained sections from cases 1, 2, 4, and 6 contained nontumoral cardiac muscle tissues.

[†]Paraffin sections from case 1 stained with *in situ* hybridization technique contained nontumoral cardiac muscle tissues.

[‡]ND, Not determined cases.

moderate to strong immunoreactivity for MEF2 in both the nucleus and cytoplasm (Figure 1D).

As shown in Table 3 and Figure 1E, the immunostaining for eHAND was variously positive in all myxoma cases, except for the case of sarcoma. Positive staining was seen in the nucleus and cytoplasm of the myxoma cells. A positive reaction for eHAND was also noted in nontumoral cardiomyocytes in the sections. Immunostaining for eHAND was also observed in the myxoma cells with ring structure (Figure 1F). Negative staining was seen in the other structures.

GATA-4 was variously positive in all myxoma cases, which showed a moderate to intense reaction (Figure 1G, Table 3). The staining of GATA-4 was mainly observed in the outer layer of the tumor cells adjacent to the capillary endothelia, whereas luminal-lining endothelial cells showed negative staining. Nontumoral cardiomyocytes in the sections showed a moderate degree of positive staining only in the nucleus (Figure 1H). Negative staining was observed in the mononuclear inflammatory cells in the myxoid stroma.

Nkx2.5/Csx immunoreactivity was observed in five myxoma cases, with a mild intensity in two and a moderate intensity in three cases. The myxoma cells from those cases showed positive staining in both the nucleus and cytoplasm (Figure 1I). Negative staining was seen in the other structures. Nontumoral cardiomyocytes in the sections showed a rather weak degree of positive staining in the nucleus and cytoplasm (data not shown).

Immunoreactivity of Nkx2.5/Csx, eHAND, GATA-4, and MEF2 with Other Tumors

Table 4 presents the results of immunostaining for Nkx2.5/Csx, eHAND, GATA-4, and MEF2 in tumors other

than cardiac myxoma. Of all of the other tumors studied, only a case of mediastinal dedifferentiated liposarcoma showed positive immunostaining for Nkx2.5/Csx and eHAND. GATA-4 was negative in all cases. MEF2 immunoreactivity was observed in the liposarcoma and malignant fibrous histiocytoma.

Light Microscopic *In Situ* Hybridization

In situ hybridization revealed that all myxoma cases except for a case of cardiac sarcoma were clearly positive for mRNA of Nkx2.5/Csx (Table 3, Figure 1J). As shown in Figure 1J, a moderately positive reaction for mRNA of Nkx2.5/Csx was noted in both the nucleus and cytoplasm of the myxoma cells. Positive staining was seen in the aggregated or singly scattered myxoma cells in the myxomatous stroma. In the case of cardiac myxoma with ring structure surrounding the dilated capillary, positive staining was also observed within the capillary-like component and the surrounding myxoma cells. Some tumor cells forming vascular-like slits or lumina were also positive for Nkx2.5/Csx mRNA. Unlike Nkx2.5/Csx, a reaction for the eHAND probe was positive in the nucleus but negative in the cytoplasm of the myxoma tumor cells (Figure 1K). The signals in the nontumoral cardiomyocytes were also observed. A negative control using sense oligonucleotide probe showed negative staining for both tumor cells and nontumoral cardiomyocytes (Figure 1L).

Transmission Electron Microscopy

In the low-magnification view of the transmission electron microscopy findings of the myxoma tissue, the myxoma cells were scattered in the abundant sparse extracellular

Figure 1. Light micrographs. Conventional histology, immunohistochemistry, and *in situ* hybridization of cardiac myxomas, sarcoma, and nontumoral cardiomyocytes. **A:** Light micrographs of cardiac myxoma (case 1), H&E stain. Cardiac myxoma cells forming abortive vascular structures or aggregated cell strands in the abundant myxoid matrix. Mononuclear inflammatory cells seen in the matrix and on the tumor cells. **B:** Immunohistochemical MEF2 distribution in cardiac myxoma (case 1). **C:** Immunohistochemical MEF2 distribution in nontumoral cardiomyocytes (case 1). **D:** Immunohistochemical MEF2 distribution in cardiac sarcoma (case 2). **E:** Immunohistochemical eHAND distribution in cardiac myxoma (case 1). **F:** Immunohistochemical eHAND distribution in cardiac myxoma with ring structure (case 3). **G:** Immunohistochemical GATA-4 distribution in cardiac myxoma (case 4). **H:** Immunohistochemical GATA-4 distribution in nontumoral cardiomyocytes (case 4). **I:** Immunohistochemical Nkx2.5/Csx distribution in cardiac myxoma (case 4). **J:** The Nkx2.5/Csx mRNA distribution demonstrated by *in situ* hybridization techniques in the cardiac myxoma (case 1). **K:** The eHAND mRNA distribution demonstrated by *in situ* hybridization techniques in cardiac myxoma (case 1). **L:** Negative control of Nkx2.5/Csx mRNA distribution demonstrated by *in situ* hybridization techniques in cardiac myxoma (case 1). Original magnifications, $\times 250$.

Table 4. Immunoreactivity of Nkx2.5/Csx, eHAND, GATA-4, and MEF2 with Other Tumors

Case	Age/sex	Diagnosis	Location	Reactivity			
				Nkx2.5/Csx	eHAND	GATA-4	MEF2
7	74 F	Malignant melanoma	Nasal cavity	—	—	—	+/-
8	56 F	Malignant melanoma	Skin	—	—	—	+/-
9	71 F	Malignant melanoma	Vulva	—	—	—	—
10	70 M	Malignant lymphoma	Lymph node	—	—	—	—
11	71 M	Malignant lymphoma	Cecum	—	—	—	—
12	56 M	Adenocarcinoma	Stomach	—	—	—	—
13	33 F	Adenocarcinoma	Lung	—	—	—	—
14	63 F	Small cell carcinoma	Lung	—	—	—	—
15	68 M	Transitional cell carcinoma	Kidney	—	—	—	—
16	54 M	Liposarcoma	Mediastinum	+	+	—	+
17	78 M	MFH [†]	Retroperitoneum	—	—	—	+

++, Intensely positive; +, moderately positive; +/-, slightly positive; —, negative.

*+, Some pleomorphic sarcoma cells are positive.

[†]MFH, Malignant fibrous histiocytoma.

matrix with some mononuclear inflammatory cells consisting of mainly macrophages (Figure 2, A and B). Densely granular depositions of hemosiderin were frequently seen both in the cytoplasm and in the extracellular stroma. Myxoma cells were spindle shaped, in either a satellite form or a round form (Figure 2, B and C). Extended cytoplasmic processes and short microvillous cellular protrusions in the cell membrane revealed the appearance of rather primitive mesenchymal cells or cultured fibroblast-like cells (Figure 2, A and B). The tumor cells were usually aggregated in the strands or small cell clusters surrounded by the irregular small capillary structures (Figure 2C). Most of the tumor cells had a well-developed rough-surfaced endoplasmic reticulum and were sometimes rich in intracytoplasmic filaments with occasional dense patches (Figure 2B). The cell junctional apparatuses of the putative primitive type and tight junctions were frequently observed (Figure 2, B and C).

There were some irregular vascular structure of capillary type that had lining endothelial cells with many Weibel-Palade bodies in the cytoplasm, well-developed pinocytotic vesicles, cell junctions, and basal lamina in the tumor tissue (Figure 2D). The luminal surface of vascular channels or slits were composed of endothelial cells and were sometimes surrounded by the cytoplasm protruding from the underlining myxoma cells (Figure 2C), which showed a rather primitive appearance with well-developed rough-surfaced endoplasmic reticulum, free ribosomes, cellular junctions between adjacent cells, and basal lamina structures around the cell membranes (Figure 2C). Myxoma cells sometimes showed an endothelium-like appearance with the continuation and surrounding position to capillary endothelia (Figure 2C).

RT-PCR Analysis of Cardiac Myxoma

We also evaluated the expression of the Nkx2.5/Csx, GATA-4, MEF2C, eHAND, and myosin light chain kinase

2v (MLC-2v) gene transcripts in cardiac myxoma from case 1. Figure 3A represents the RT-PCR analysis of Nkx2.5/Csx, GATA-4, MEF2C, eHAND, and MLC-2v. Cardiomyocytes and cardiac myxoma cells, but not hepatocytes, expressed Nkx2.5/Csx, eHAND, MEF2C, and GATA-4 gene transcripts. MLC-2v represents a product suggesting terminal cardiac muscle differentiation, however, cardiac myxoma cells did not express MLC-2v. Normal heart tissue or liver tissue was used as either a positive or a negative control, respectively. The negative controls produced no visible band.

Nested RT-PCR from Formalin-Fixed Paraffin-Embedded Sections

We further performed RT-PCR from formalin-fixed, paraffin-embedded sections. As shown in Figure 3B, the amplified fragment of the Nkx2.5/Csx transcript was observed in five of six cases of myxoma except for the case of cardiac sarcoma. Whereas, three of six cases exhibited a specific eHAND signal of 174-bp transcript. β -actin transcripts were amplified in all of the samples and served as a control for amplifiable mRNA.

Discussion

The conventional histopathological features of cardiac myxoma were not definitive to conclude the histogenesis or to identify the specific cell lineage by the phenotypic characterization (Figure 1A), as has been described to date. Electron micrographs also failed to demonstrate any evidence of myogenic differentiation characteristic of organized sarcomeres or mature myotubes (Figure 2, A to D).

The recent discovery of tissue-specific transcription factors involved in morphogenesis has allowed us to

Figure 2. Transmission electron micrographs of the cardiac myxoma from case 1. **A and B:** Spindle-shaped myxoma cells forming cell strands in the myxoid stroma. **Arrow**, Cellular junctions between the adjacent tumor cells; **arrowhead**, rich intracytoplasmic filaments with a subplasmalemmal dense patch; **M**, macrophages with many lysosomes and hemosiderin deposition in the cytoplasm. **C and D:** Tumor cells forming a capillary in the myxoma tissue. Tumor cells aggregated forming cell clusters with many short villous cytoplasmic processes, many cellular junctions, and well-developed pericellular lamina. **Cap**, Tumor cells continuing the structure of a capillary blood vessel showing the lumen; **arrow**, Weibel-Palade bodies; **arrowhead**, cellular junctions between the adjacent cells; **L**, well-developed lamina around the cells. Original magnifications: $\times 2,500$ (**A**); $\times 4,700$ (**B**); $\times 16,000$ (**C**); $\times 32,000$ (**D**).

Figure 3. RT-PCR analysis of cardiomyocyte-specific transcription factors. **A:** RT-PCR analysis of Nkx2.5/Csx, eHAND, MEF2C, GATA-4, and MLC-2v transcript in cardiac myxoma from case 1. Total RNA was isolated from heart (H), liver (L), and cardiac myxoma (C) from case 1. After DNaseI treatment, RT-PCR was performed. The heart and liver were used as positive and negative controls. M, Φ XHaeIII molecular size marker. Cardiomyocytes and cardiac myxoma cells, but not hepatocytes, expressed Nkx2.5/Csx, eHAND, MEF2C, and GATA-4. Cardiac myxoma cells did not express MLC-2v. **B:** Nested RT-PCR analysis of Nkx2.5/Csx and eHAND. Total RNA was isolated from formalin-fixed, paraffin-embedded sections of the cardiac myxoma and sarcoma from cases 1 to 6, and nested RT-PCR was performed. The expression of Nkx2.5/Csx transcripts was detected in all samples, whereas eHAND transcripts were positive in three of six samples. M1 and M2 represent the Φ XHaeIII and 200-bp molecular size markers, respectively. Samples were identified according to the case's number.

better understanding the molecular events that underlie the establishment of specific cell differentiation. In view of the differentiation and development of cardiomyocytes, it is thought that the cardioblast is formed after the expression of Nkx2.5/Csx gene, and thereafter develops into the myotube in the presence of MEF2C.^{9,11,12}

In the present study, cardiac myxoma cells expressed various amounts of cardiomyocyte-specific transcription factor, Nkx2.5/Csx, eHAND, GATA-4, and MEF2 gene products at the mRNA and protein levels. Because of the cardiomyocyte-specific expression of these transcription factors in the adult, the expression of homeobox gene, Nkx2.5/Csx in myxoma cells is highly suggestive of its cardiomyogenic lineage. As reported in an earlier study, GATA-4 expression is predominantly restricted to the developing heart and gonads and is present at high levels throughout the myocardium and endocardium in the postnatal heart.¹⁵ eHAND is also expressed on the

left side of the heart, in the neural crest-derived cardiac outflow tract and aortic arch arteries.¹⁶

MEF2 is a muscle-specific DNA-binding protein of myogenic helix-loop-helix transcription factor of myogenin/MyoD class, whose expression is preferential in skeletal and cardiac muscle cells.¹³ The MEF2 antibody reacts with both MEF2A and MEF2C. Desmin and HHF35 are commonly used sensitive markers of myogenic differentiation such as rhabdomyosarcoma or leiomyosarcoma, whereas, MyoD has been reported to be useful for discriminating striated muscle cell tumors from smooth muscle cell tumors, or for detecting myogenic differentiation, especially in cases with a low degree of differentiation.²¹ The immunostaining of MEF2 was variously positive in all cases, irrespective of the histological characteristics. Cardiac sarcoma cells, which were not immunoreactive with MyoD, desmin, or HHF35 (data not shown), however, showed moderate to strong immunoreactivity for MEF2, thus suggesting that cardiac sarcoma cells might present a different type of myogenic differentiation from skeletal or smooth muscle cells.

Immunohistochemistry also revealed that a slightly to intensely positive reaction was also observed with eHAND, GATA-4, and Nkx2.5/Csx in all myxoma cases. It is noteworthy that all myxoma cases showed a variable but significant degree of immunoreactivity for these cardiomyocyte-specific transcription factors independent of the histological pattern, vascular channels, degenerative changes or the myxomatous background. The expression of these transcription factors may be used as a sensitive marker for the diagnosis of cardiomyogenic differentiation. Interestingly, some parts of cardiac sarcoma tissue presented similar histopathological features such as myxoma, which is considered to be a malignant counterpart of cardiac myxoma, however, the expression patterns of these cardiomyocyte-specific transcription factors are quite different. On the other hand, the immunohistochemistry in other tumors revealed that, with the exception of a rare case of mediastinal liposarcoma, their expression appeared to be relatively specific for cardiac myxoma. However, the number of cases tested herein is small and limited, and further immunohistochemical studies are needed to enhance the understanding and the diagnostic utility of these cardiomyocyte-specific transcription factors in clinical specimens.

It is also surprising to find that *in situ* hybridization or RT-PCR for the cardiac homeobox gene, Nkx2.5/Csx showed all cases of cardiac myxoma exhibiting mRNA of Nkx2.5/Csx. Although a certain number of light microscopic, ultrastructural, and immunohistochemical studies have been published to date, only a few analyses regarding mRNA expression in myxoma cells have yet been reported. It is generally thought that RNA is easily destroyed by ubiquitous RNase and may also degrade during the course of tissue processing and the storage of specimens. Nevertheless, under the right conditions, RNA can be preserved for years in archival specimens.^{22,23}

Myxoma cells may have the ability to differentiate into cardiomyogenic cells and their positivity for Nkx2.5/Csx, GATA-4, eHAND, and MEF2, and negativity for MLC-2v may thus be indicative of immature cells that have not yet

expressed the characteristic phenotype of cardiomyocytes. The cells giving rise to the tumors are considered to be mesenchymal cells that persist as embryonal residues^{4–7} during septation of the heart and may later differentiate into cardiomyocyte precursor cells along with endothelial,^{3–6} fibroblastic,⁵ neurogenic,^{5–7} and smooth muscle cells.^{3–5} We agree that the identification of tumor origin by specific antigen expression may be misleading, because the phenotypic expression in neoplasia can be variable and does not necessarily reflect its origin. However, the positivity for various cardiomyocyte-specific transcription factors in myxoma cells clearly suggests that cardiac myxoma is a neoplasm arising from mesenchymal cells with a cardiomyogenic lineage.

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