Thrombomodulin, calretinin and c-kit (CD117) expression in cardiac myxoma

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Summary. The immunohistochemical profile of cardiac myxoma has been debated. The tumor is thought to be derived from multipotential undifferentiated mesenchymal cells. A consistent marker for this tumor has not been found. In this article an immunohisto-chemical study of 23 cardiac myxomas was accomplished. This study comprised the immuno-reactivity of the tumors for thrombomodulin, calretinin and and c-kit (CD117). To the best of our knowledge, thrombomodulin and c-kit have not been tested in cardiac myxoma. Calretinin expression has been recently demonstrated in cardiac myxoma, although this finding has not been yet validated. Surface lining cells, tumor vascular endothelium, cells around the vascular slits and stromal cells embedded in the myxoid matrix were assessed independently. All tumors showed reactivity for thrombomodulin in the surface cells and in the endothelium of neoplastic vessels. 82.6% of cardiac myxomas expressed thrombomodulin in the stromal cells and 69.6% of the tumors were reactive in the perivascular cells. 73.9% of cardiac myxomas expressed calretinin in the stromal cells and in the perivascular cells. All myxomas were negative for c-kit. Thrombomodulin and calretinin may be important diagnostic aids for cardiac myxoma. Cardiac myxoma cells do not express embryonic/fetal endothelial antigens.

Key words: Cardiac myxoma, Thrombomodulin, Calretinin, Immunohistochemistry

Introduction

Myxoma is the most common primary cardiac tumor in adults. This neoplasm may arise in any of the four chambers, or rarely on the valves. Cardiac myxoma is a benign neoplasm of controversial histogenesis that occurs exclusively on the endocardial surface. The immunohistochemical profile of this tumor is debatable and of little use in the differential diagnosis (Burke and Virmani, 1996). Endothelial, muscle, and neural markers (including S100 protein) have been reported with variable results and marked discrepancy among authors (Burke and Virmani, 1996).

In this article, a detailed evaluation of 23 cases by immunohistochemistry was accomplished. The immunohistochemical studies are reported using antibodies to thrombomodulin, calretinin and c-kit proto-oncogene product (CD117). Thrombomodulin, a regulator of intravascular coagulation, was first demonstrated in vascular endothelium, whereby it seems interesting to assess its expression in an angioformative tumor, like cardiac myxoma. Calretinin, a member of a large family of calcium binding cytoplasmic proteins, to which the S100 protein also belongs, has been recently described in cardiac myxomas in a study that has not been yet validated (Terraciano et al., 2000). C-kit (CD117), a tyrosine kinase growth factor receptor for stem cell factor, is expressed in embryonic/fetal endothelium but not in adult and neovascular endothelial cells (Miettinen et al., 2000). As far as we are aware thrombomodulin and c-kit have not been used before in this tumor. The purpose of this study was to find a more consistent marker for cardiac myxoma.

Materials and methods

The study comprised 23 consecutive patients with cardiac myxomas surgically treated from 1973 until March 2000 at our hospital. Formaldehyde-fixed and paraffin-embedded blocks from 23 resected operative specimens were used for immunohistochemical study.

For light microscopic studies, the slides were stained with hematoxylin-eosin.

For the immunohistochemical study the 5 μm-sections, mounted on precoated slides, were stained by using the EnVision+ method (Dako, Glostrup, Denmark) and a TechMate 500 automated immunostainer (BioTek, Santa Barbara, CA, USA). Diaminobenzidine (Dako) was used as a chromogen. To block endogenous

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peroxidase activity, sections were treated with 0.3% H_2O_2 for 30 minutes. The primary antibodies utilized were thrombomodulin (clone 1009, Dako, dilution 1:50), calretinin (polyclonal, Zymed, San Francisco, CA, USA, 1:100), and c-kit (polyclonal, Dako, 1:100). Prior to the staining with calretinin and c-kit sections were boiled for two minutes in 10mM sodium citrate buffer, pH 6.0, using a pressure cooker. For the thrombomodulin heat-epitope retrieval was not used. The slides were counterstained with Mayer's hematoxylin (Merck, Darmstadt, Germany) and dehydrated. To evaluate the specificity of the antibodies positive and negative tissues were used as controls. The grading of the immuno-staining was accomplished on a sliding scale of 0 (negative), 1+ (focal staining) to 2+ (diffuse staining). Cells forming perivascular ring structures and cells
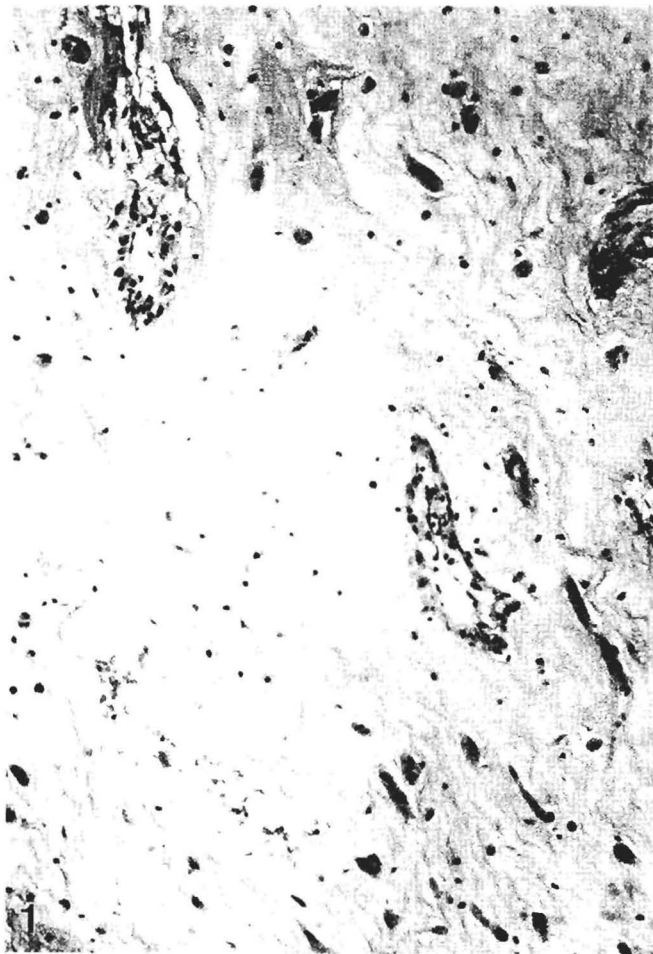

Fig. 1. Cardiac myxoma. Myxoma cells form rings around blood vessels and appear as individual elements, short cords, or syncytia embedded in a myxoid matrix. Hematoxylin and eosin, x 40

embedded in the myxoid matrix were assessed independently.

For comparison five myxoid mural cardiac thrombi were included in the study.
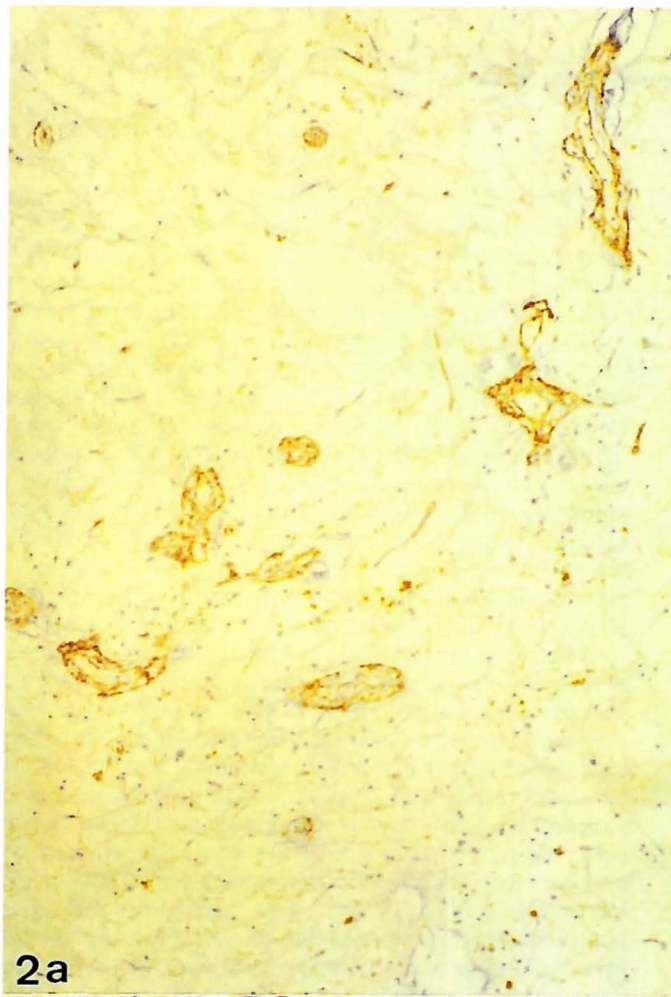
Results

Of the 23 patients 15 were females and 8 males with a mean age of 51 ± 13 years (range 23-71 years). All 23 cases arose from the atrial septum. Seventeen tumors were located in the left atrium, five in the right atrium, and one in both atria.

Histologically, the tumors consisted of a myxoid matrix composed of an acid mucopolysaccharide-rich stroma in which were embedded polygonal-, round-, spindle-, or star-shaped cells arranged singly, in small groups, forming branching cords, or in tufts. Rings of cells one to several layers thick surrounding a blood vessel were seen in all the cases (Fig. 1). One case contained glandular structures lined by mucin-laden cells. The myxoma surfaces were usually lined by a monolayer of flattened cells resembling endocardium, but sometimes the covering cells were plump with focal clustering or forming multiple layers. Immuno-histochemical staining for thrombomodulin showed membrane reactivity in positive cases. The endothelial cells of the non-tumoral vessels and the endocardial cells of the base of attachment of the myxoma showed positivity and were utilized as internal controls. All tumors displayed reactivity for thrombomodulin in the surface cells (including multiple cell layers and invaginations) and in the endothelium of neoplastic vessels. The results of immunohistochemical staining are summarized in Table 1. The perivascular cells were positive in 16 (69.6%) cases (Fig. 2a) and negative in 7 (30.4%) cases. The stroma cells showed focal positive staining in 10 (43.5%) out of the 23 cases; and in 9 (39.1%) the positivity was diffuse (Fig. 2b). Thus, total positivity of stromal cells was 82.6%. The glandular component of one myxoma was negative.

Immunohistochemical staining for calretinin showed nuclear and cytoplasmic reactivity in positive cases. The adipocytes of the base of attachment of the myxoma showed focal positivity and acted as internal controls. Staining for calretinin was positive, mostly diffuse, in the perivascular cells in 17 (73.9%) out of 23 cases (Fig. 2c). All endothelial cells were negative. Stromal cells showed positivity, mostly diffuse, in 17 (73.9%) out of 23 cases (Table 1) (Fig. 2d). Myxoma lining cells displayed focal positive staining in four (17.4%) out of 23 cases. The glandular component of one myxoma was negative.

Eleven (47.8%) out of those 23 cases showed simultaneous positive staining for thrombomodulin and calretinin in perivascular and stromal type of cells (Table
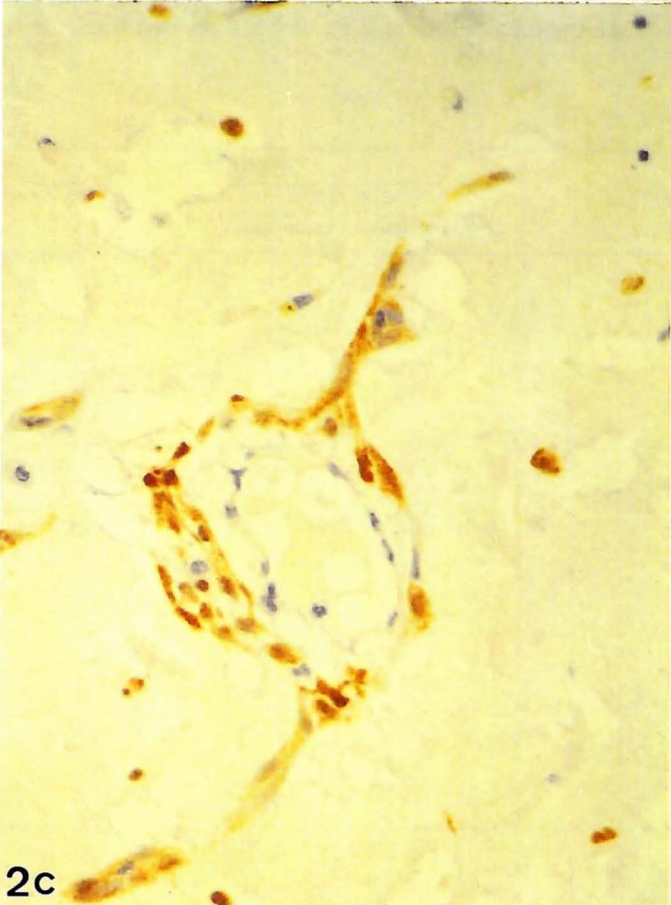
Fig. 2. Positive reactivity for thrombomodulin in the perivascular cells (**a**); and in the stromal cells (**b**). Positive staining for calretinin in the perivascular cells (**c**); and in the perivascular and stromal cells (**d**). a, x 25; b, c, x 100; d, x 40
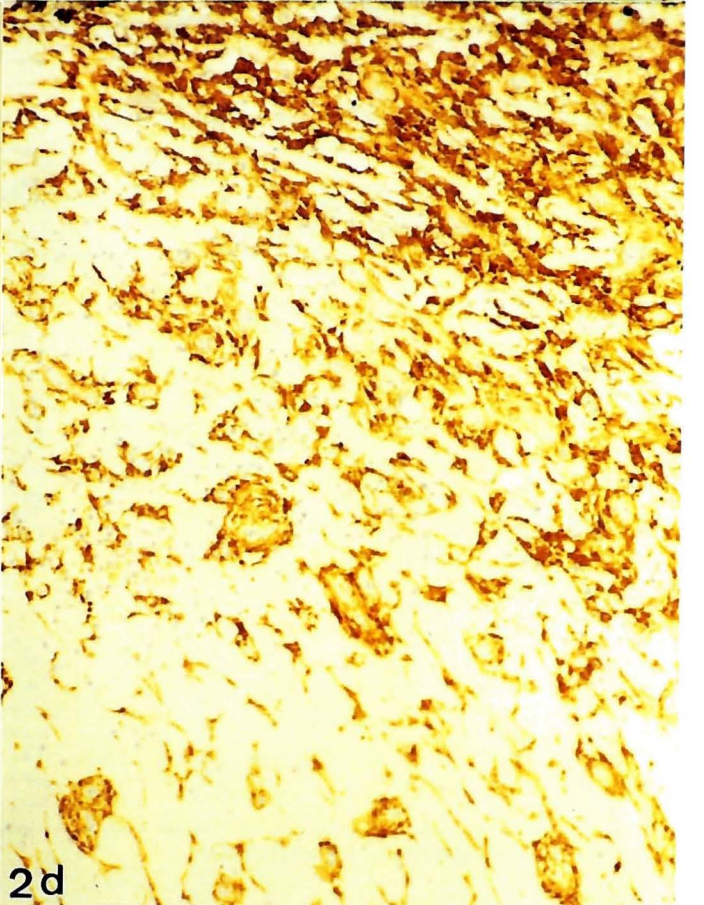
2a
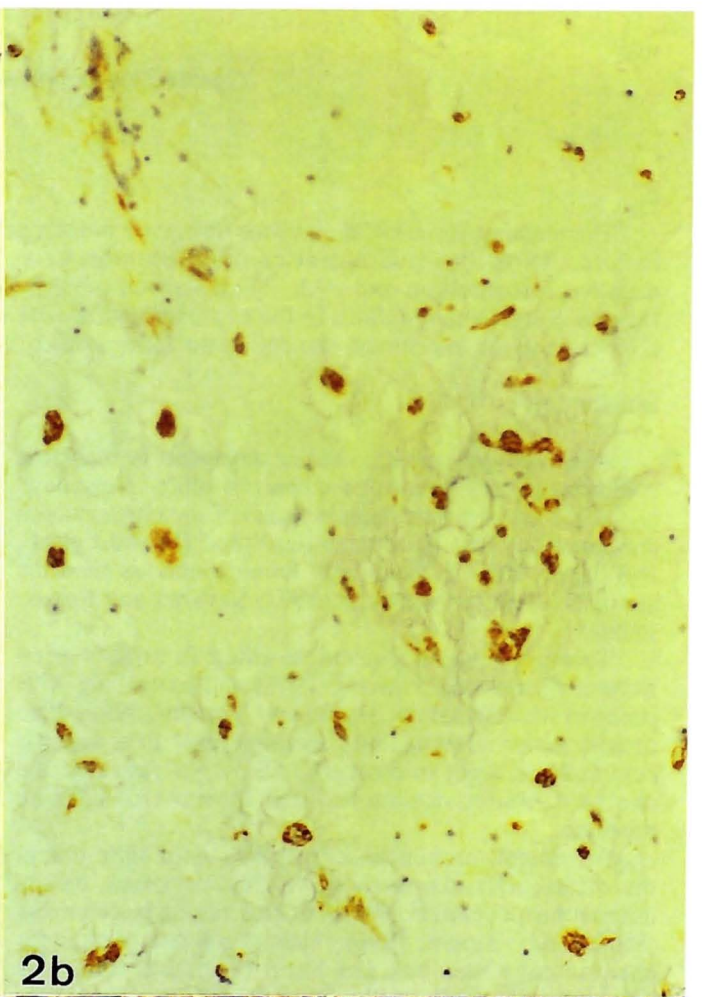
2b

2c

2d

1).

The staining for c-kit in all cases displayed negative results. All of the five mural cardiac thrombi were negative for calretinin and c-kit. They showed positive staining for thrombomodulin in the capillary endothelial cells close to the attachment site and in the lining cells.

Discussion

Although infrequent, cardiac myxoma is the most common primary tumor of the heart in adults. Nowadays it is regarded as a neoplasm because of the chromosome abnormalities that have been described (Dewald et al., 1987), and thrombogenic and hamartomatous theories are discredited (Salyer et al., 1975; Suvarna and Royds, 1996).

This neoplasm occurs more often in middle aged women (Burke and Virmani, 1996) and more than 75% occur in the left atrium (Burke and Virmani, 1993). The present series shows similar demographic data and the conventional light microscopic study has revealed the characteristic histopathological features of cardiac myxoma.

In general, immunohistochemistry is of little use in the differential diagnosis of cardiac myxoma, as the immunohistochemical profile of this tumor is debatable (Burke and Virmani, 1996). Thus, a reliable marker for cardiac myxoma has not been reported. There is agreement on the facts that surface cells and vascular-like lumen-lining cells within myxomas are reactive for endothelial markers (Morales et al., 1981; Boxer, 1984; McComb, 1984; Landon et al., 1986; Tanimura et al., 1988; Curschellas et al., 1991; Burke and Virmani, 1993, 1996; Farrell et al., 1996) and that the tumors consistently express vimentin (Govoni et al., 1988; Johansson, 1989; Burke and Virmani, 1993, 1996). Epithelial, muscle and neural markers have shown variable results in myxoma cells (Burke and Virmani, 1996). Since some myxomas contain cells that express S100 protein, protein gene product 9.5, neuron-specific enolase (Krikler et al., 1992; Pucci et al., 2000) and synaptophysin (Krikler et al., 1992), it has been suggested that these tumors originate from endocardial sensory nerve tissue (Krikler et al., 1992). One report has demonstrated that cardiac myxoma is rich in factor XIIIa positive dendrophages and CD34 positive mesenchymal cells, whose presence suggested abnormal organizing thrombus-like differentiation (Berrutti and Silverman, 1996). Interleukin 6 has also been demonstrated by immunohistochemistry in a high percentage (80%) of cardiac myxomas, and it seems to be involved in the immunologic abnormalities of patients with this tumor (Kanda et al., 1994). On the other hand, myxoma cells do not express specific markers for histiocytic differentiation (Burke and Virmani, 1993, 1996). The variable and even contradictory immunohistochemical results in cardiac myxoma suggested to Burke and Virmani (1996) that this tumor arises from primitive stromal cells that have the capacity to differentiate along many cell lines.

Table 1. Summary of the immunohistochemical study of the cardiac myxomas.

CASE no.	THROMBOMODULIN			CALRETININ		
	Ring structures		Stromal cells	Ring structures		Stromal cells
	Endothelium	Outer layer		Endothelium	Outer layer	
1	++	+	+	-	+	+
2	++	-	-	-	++	++
3	++	-	-	-	+	+
4	++	++	++	-	++	++
5	++	+	+	-	-	-
6	++	+	+	-	+	+
7	++	+	+	-	-	-
8	++	-	+	-	++	++
9	++	-	+	-	++	++
10	++	++	++	-	-	-
11	++	-	-	-	-	-
12	++	++	++	-	-	-
13	++	++	++	-	-	-
14	++	-	-	-	++	++
15	++	++	++	-	++	++
16	++	++	++	-	+	+
17	++	-	+	-	++	++
18	++	+	+	-	+	+
19	++	+	+	-	+	+
20	++	+	+	-	+	+
21	++	++	++	-	++	++
22	++	++	++	-	++	++
23	++	+	++	-	++	++

-: negativity; +: focal positivity; ++: difuse positivity

Thrombomodulin is a 75 kDa cell surface glycoprotein involved in the regulation of intravascular coagulation (Esmon, 1989; Dittman and Majerus, 1990). Initially, the expression of thrombomodulin was thought to be limited to vascular endothelium, but it is now accepted that many other cells express this glycoprotein, including squamous epithelial cells, syncytio-trophoblasts, mesothelial cells, synovial cells, meningeal cells, activated smooth muscle cells, white blood cells, and platelets (Dittman and Majerus, 1990; Jackson et al., 1995; Ordóñez, 1998). Thrombomodulin expression has also been documented in a variety of tumors including squamous cell carcinomas of the skin, esophagus and lung, transitional cell carcinoma, trophoblastic tumors, mesotheliomas, angiosarcomas, and in various types of adenocarcinomas, including pulmonary adeno-carcinomas (Jackson et al, 1995; Ordóñez, 1998).

In this study, we have immunohistochemically demonstrated that more than 82% of cardiac myxomas express thrombomodulin in the stromal cells and more than 69% of the tumors are reactive in the perivascular cells. Furthermore, all tumors displayed reactivity for thrombomodulin in the surface cells and in the endothelium of neoplastic vessels. These findings suggest that the constituent cells of atrial myxoma are involved in the local regulation of intravascular coagulation. Thrombomodulin is a potent activator of protein C that acts by a mechanism whereby the protease thrombin is converted from a procoagulant to an anticoagulant (Dittman and Majerus, 1990). It is interesting to contrast the expression of thrombomodulin, an anticoagulant protein, and the reported expression of XIIIa factor, a procoagulant protein, in the stromal histiocytic cells of cardiac myxomas (Berrutti and Silverman, 1996); which suggests that a delicate control of coagulation take place in these tumors. Thrombomodulin has a limited use as a marker for the differential diagnosis of myxoma, as the antibody is also expressed in mural cardiac thrombi. However, the staining pattern showing positivity in the perivascular and stromal cells may help in diagnosis.

Calretinin is a 29 kDa Ca^{2+} binding protein that is expressed in neurons of the central and peripheral neural system, mesothelial cells, adipocytes, muscle spindle, superficial keratinizing layer of the pilar infundibulum, eccrine glands, convoluted tubules of the kidney, mastocytes, Leydig and Sertoli cells, rete testis, endometrial stroma, surface ovarian epithelium, ovarian stroma cells, theca cells, hilus cells of the ovary, rete ovary, and keratinizing thymic epithelial cells (Dei Tos and Doglioni, 1998). Calretinin, like S100 protein belongs to the EF-hand family of calcium binding proteins. Calretinin is also expressed transiently in embryonic mesenchymal cells (Gangji et al., 1997). Calretinin is found localized in association with the microtubules of the mitotic spindle of WiDr tumor cells cultivated in vitro (Gotzos et al., 1992) and it is thought that it can play a role in the neoplastic proliferation of the cells expressing it. Calretinin expression has also been documented in a variety of tumors including neoplasms with neuronal differentiation, mesothelioma, and adenocarcinoma of colon. Interestingly, calretinin is expressed by most undifferentiated colorectal adenocarcinomas, but only by a limited number of cells in well-differentiated tumors (Gotzos et al., 1999). Recently, it has been published that calretinin can be used as a marker for cardiac myxoma, because of a study in which calretinin expression was identified in 100% of cases from a series of 24 tumors (Terracciano et al., 2000). Calretinin is also regarded as a tool for the differential diagnosis of cardiac myxomas with mural myxoid thrombi, which are negative for this marker (Terracciano et al., 2000).

In our series, we have immunohistochemically demonstrated that more than 73% of cardiac myxomas express calretinin in the perivascular and stromal cells. This fact can be correlated with an undifferentiated state of the myxoma cells reactive for calretinin. Thus, in concordance with the observations on WiDr (Gander et al., 1996) and HT29 (Cargnello et al., 1996) cells, calretinin can be involved in maintaining the cardiac myxoma cells in an undifferentiated state, and sustaining the progression of the proliferative cell cycle. As in the cases studied by Terrracciano et al. (2000), calretinin was negative in all our cases of mural thrombi.

C-kit gene encodes for a tyrosine kinase growth factor receptor for stem cell factor (or mast cell growth factor). C-kit protein is expressed in hematopoietic stem cells, mast cells, early trophoblast, germ cells, basal epithelial cells, luminal epithelium of breast, melanocytes, and the interstitial cells of Cajal of the gastrointestinal tract (Vliagoftis et al., 1997). Tumors reactive for c-kit (CD117) include gastrointestinal stromal tumors, small cell lung carcinoma, adenoid cystic carcinoma, mastocytosis, seminoma/dys-germinoma, and angiosarcoma (Tssura et al., 1994; Miettinen et al., 2000).

In our study cardiac myxomas do not express c-kit protein. This result is concordant with the fact that c-kit is expressed in embryonic/fetal but not in adult and neovascular endothelial cells (Miettinen et al., 2000). The c-kit reactivity in angiosarcoma may represent oncofetal expression of the vascular malignant cells (Miettinen et al., 2000). Our results suggest that the cells of cardiac myxoma do not express embryonic/fetal endothelial antigens.

In this study we have validated the immunoreactivity of cardiac myxoma cells for calretinin, as well as its utility in the differential diagnosis of cardiac myxomas with mural thrombi. We have also added a novel marker for cardiac myxoma, thrombomodulin, which is positive in the majority of the cases. Both of these antibodies should be considered in the immunohistochemical differential diagnosis of neoplasms.

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