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ORIGINAL ARTICLE – CANCER RESEARCH

Heart myxoma develops oncogenic and metastatic phenotype

Silvia Cecilia Pacheco-Velázquez[1] · Juan Carlos Gallardo-Pérez[1] · Daniel Díaz[2] · Alhelí Adán-Ladrón de Guevara[1] · Diana Xochiquetzal Robledo-Cadena[1] · Emma Saavedra[1] · Luz Ruiz-Godoy[3] · Luis Roberto Jimenez-Hernández[4] · Jesús Vargas-Barrón[4] · José Luis Aguilar-Ponce[3] · Sara Rodríguez-Enríquez[1]

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Abstract

Purpose Heart myxomas have been frequently considered as benign lesions associated with Carney's complex. However, after surgical removal, myxomas re-emerge causing dysfunctional heart.

Methods To identify whether cardiac myxomas may develop a metastatic phenotype as occurs in malignant cancers, a profile of several proteins involved in malignancy such as oncogenes (c-MYC, K-RAS and H-RAS), cancer-associated metabolic transcriptional factors (HIF-1α, p53 and PPAR-γ) and epithelial–mesenchymal transition proteins (fibronectin, vimentin, β-catenin, SNAIL and MMP-9) were evaluated in seven samples from a cohort of patients with atrial and ventricular myxomas. The analysis was also performed in: (1) cardiac tissue surrounding the area where myxoma was removed; (2) non-cancer heart tissue (NCHT); and (3) malignant triple negative breast cancer biopsies for comparative purposes.

Results Statistical analysis applying univariate (Kruskal–Wallis and Dunn's tests) and multivariate analyses (PCA, principal component analysis) revealed that heart myxomas (7–15 times) and myxoma surrounding tissue (22–99 times) vs. NCHT showed high content of c-MYC, p53, vimentin, and HIF-1α, indicating that both myxoma and its surrounding area express oncogenes and malignancy-related proteins as occurs in triple negative breast cancer.

Conclusions Based on ROC (receiver operating characteristics) statistical analysis, c-MYC, HIF-1α, p53, and vimentin may be considered potential biomarkers for malignancy detection in myxoma.

Keywords Hearth myxoma · Malignancy · Metastasis · Metastatic biomarkers · Oncogenic profile

Introduction

Myxomas are the most common benign primary heart tumors followed by lipomas and papillary fibroelastomas (Singh et al. 2015). They can be developed in the four heart chambers (Vargas-Barron et al. 1991), being left atrium (80%) the most common site (Tarelo-Saucedo et al. 2016; Kothari et al. 2016). The disorder is transmitted within families in an autosomal dominant manner with higher occurrence in young women (Siltanen et al. 1976). Myxoma is also a primary component of the Carney's complex, a multiple familial neoplasia/lentiginous syndrome (Carney 1985; Vargas-Alarcón et al. 2008).

Although its origin is not clear, it is hypothesized that heart myxoma arises from sub-endothelial cells or primitive cells residing in the *fossa ovalis* and surrounding endocardium (Burke et al. 2007). Additionally, it has been proposed that Herpes simplex virus DNA infection may be involved in the pathogenesis of atrial myxoma (Pateras et al. 2012).

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✉ Sara Rodríguez-Enríquez
saren960104@hotmail.com;
sara.rodriguez@cardiologia.org.mx

[1] Departamento de Bioquímica, Instituto Nacional de Cardiología, Juan Badiano No. 1. Col Sección XVI, Tlalpan, Mexico City, Mexico

[2] Centro de Ciencias de la Complejidad (C3), UNAM, Mexico City, Mexico

[3] Banco de Tumores, Instituto Nacional de Cancerología, Mexico City, Mexico

[4] Dirección de Investigación, Instituto Nacional de Cardiología, Mexico City, Mexico

It has been documented that after myxoma emerges, it may be fragmented, detached and disseminated to other organs (spleen, pancreas, liver, and kidney), being the central nervous system the most affected (> 50% of cases) (Lee et al. 2007; Pinede et al. 2001; Blackmon et al. 2010; Smith et al. 2012). Myxoma fragmentation and migration is promoted by systemic embolic episodes (Smith et al. 2012) in more than 30% of patients with left atrial myxoma (Fyke et al. 1985).

Myxoma promotes organ dysfunction (blood flow obstruction, embolism) and other heart-related symptoms (fever, fatigue, weight loss) (Pérez-Andreu et al. 2013). Thus, it should be surgically removed as the first treatment option. However, recurrence rate of myxoma is 2–4% (Reynen 1995; Gošev et al. 2013) being more frequent (12–25%) in the familial myxoma type, especially in patients with Carney syndrome (Carney 1985). Recurrent myxomas usually appear at or near the site of the primary tumor, in other cardiac chambers or even at distant locations (Roldan et al. 2000; Rathore et al. 2008) usually during the 4 years after surgical excision, but they can emerge within a few months to several years (10–12 years) (Reynen 1995; Roldan et al. 2000). It has been reported that recurrent myxomas develop aggressive phenotypes, switching from a benign to malignant tumor (Hannah et al. 1982; Hou et al. 2001). Myxoma recurrence may be related to an inadequate tumor resection during the surgical procedure (Mendoza et al. 2007); but also, to the development of totipotent multicentricity and/or metastatic phenotype (Becker et al. 2008).

Regarding metastasis, contradictory information has been found in the literature. For example, one study revealed low or null presence of proliferation, metastatic potential and oncogene markers (Ki-67, Bcl-2, and Rb-1 proteins) in a cohort of ten cases of cardiac myxoma, indicating a weakly proliferative lesion with scarce metastatic potential (Suvarna and Royds 1996). Unfortunately, in this last study only three proteins were analyzed. Other studies rather point the contrary: (a) It has been found that some cardiac myxomas may develop a sarcoma-like phenotype inducing brain metastasis (Todo et al. 1992); (b) in other benign-type heart tumors as papillary fibroelastomas, a high content in the metastatic biomarker oncogene K-RAS (mutated isoform) (Wittersheim et al. 2017) was found suggesting a malignant phenotype acquisition (Becker et al. 2008) and (c) oncogenes as c-MYC have been implicated in the switch from myocyte proliferation to terminal hearth differentiation (Jackson et al. 1990). However, there are no available studies showing other malignant profile biomarkers in heart myxomas rather than the typical transcription factors K-RAS and c-MYC.

Therefore, in the present study and for first time a targeted proteomic profiling of several well-documented proteins (at least 11) related to malignancy cancer progression (epithelial–mesenchymal transition, invasiveness, oncogenes, tumor metabolism) were analyzed in human myxomas. The development of a biomarker panel for malignancy diagnosis in human heart myxomas would help to establish a medical prevention protocol against myxoma recurrence.

Materials and methods

Tissue collection

The present cross-sectional study (from 2015 to 2017) was approved by the Committees of Ethics and Research of *Instituto Nacional de Cardiología Ignacio Chávez, México* (*INCAR*) (https://www.cardiologia.org.mx/comite_etica_investigacion/pdf/constancia_comite_bioetica.pdf) and included the evaluation of seven myxoma samples from 6 patients (two were obtained from one patient) diagnosed with atrial myxoma without previous clinical treatment (Supplementary Table S1). For comparative purposes (a) heart tissue samples from the surrounding myxoma area were collected, (b) normal heart tissue derived from 7 patients (age from 18 to 66 years, mean 41 ± 17 years) undergoing heart surgery not associated to myxoma removal and (c) diagnosed human triple negative breast cancer (TNBC) tissue were also analyzed (Supplementary Table S1). The medical procedure for TNBC handling and patient care was approved by the Committees of Ethics and Research of *Instituto Nacional de Cancerología, México* (*INCAN*) (http://incan-mexico.org/incan//pub/investigacion/bioetica/Anexo7.pdf). Both INCAR and INCAN Committees of Ethics and Research were supported by a patient's informed consent prepared according to the Declaration of Helsinki. Seven was the minimum sample size in pilot and prospective studies based on (1) the α level = 0.05; (2) low error margin = 3% and (3) low disease population frequency = 0.0016, according to the Cochran's formulation (Bartlett et al. 2001). All the biopsy samples were stored in liquid nitrogen until their use as described before (Pacheco-Velázquez et al. 2014).

Western blot analysis

Biopsies were resuspended and homogenized with a Teflon-pestle in 0.4 mL of 25 mM Tris–HCl buffer, 1 mM PMSF, 1 mM EDTA and 5 mM DTT and centrifuged at 10,000 rpm for 30 min at 4 °C (Pacheco-Velázquez et al. 2014). The supernatants were collected, and the protein concentration was determined by the Lowry assay (Lowry et al. 1951). Protein samples (50 µg) were resuspended in loading buffer with 10% glycerol, 2% SDS and 5% β-mercaptoethanol and they were separated by SDS-PAGE in 10 or 12.5% polyacrylamide gels. Afterward, proteins were blotted into PVDF membranes and incubated overnight with the following antibodies: anti-HIF-1α (sc-13515), -c-MYC (sc-70464), -p53 (sc-126), -PPAR-γ

(sc-166731), -fibronectin (sc-8422), -vimentin (sc-7557), -β-catenin (sc-7963) and -α-tubulin (sc-5286) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 1:1000 dilution; anti -H-RAS (sc-53958), -K-RAS (sc-30), -SNAIL (sc-10433), -MMP-9 (sc-12759) (Santa Cruz Biotechnology) at 1:500 dilution. Bands of hybridization were detected with the corresponding secondary antibodies, and the horseradish peroxidase reaction as previously described (Gallardo-Pérez et al. 2014). Densitometry analysis was carried out using the Scion Image software (Scion; Walkersville, MD, USA). Normalization of all samples was performed against its respective loading control (α-tubulin) which was considered as 100% (Pacheco-Velázquez et al. 2014).

Statistical analyses

Significant differences in the evaluated protein contents among the samples were analyzed using the non-parametric (NPA), Kruskal–Wallis test followed by Dunn's multiple comparison test (corrected for multiple comparison using statistical hypothesis testing) statistical analyses (Pacheco-Velázquez et al. 2014; Dunn 1964). The NPA, univariate analyses and graphical data were carried out using the Microsoft SPSS v. 21 (IBM, Chicago, IL, USA), Microsoft Origin Pro 8 (OriginLab Corporation, Northampton, MA, USA) and Prism 7 (GraphPad Software, La Jolla, CA, USA) software, respectively. To validate these results and for a comparative visualization of the protein expression pattern among each assayed group, univariate analysis applying star plots (Ibarra-Coronado et al. 2015), and Z-score value's heatmaps (Diaz et al. 2018) were performed. The star plot is a graphical method of displaying multivariate data, which allows the direct comparison among groups (i.e., myxoma, surrounding, TNBC, and NCHT) of three or more quantitative variables (i.e., the expression of each protein) represented on axes starting from the same point (Ibarra-Coronado et al. 2015; Aguirre-Benítez et al. 2017). For the identification of the oncogenic pattern in myxoma samples, multivariate analysis (principal component analysis) using a covariance matrix derived from protein band intensities normalized against loading control was performed (Chambers et al. 1983). The PCA and graphical data were carried out using the SAS University Edition (SAS Institute Cary, NC, USA) software. Receiver operative characteristic (ROC) curves were also determined and analyzed for the identification of the cut-off values for each evaluated protein. ROC curve analysis was performed with SPSS v. 21 (IBM, Chicago, IL, USA) software. All statistical tests were performed at a significance level of 0.05 as reported for human biopsy studies (Abba et al. 2010). Values are expressed as the mean ± S.D.

Results

This study analyzed 7 samples from a cohort of 6 patients diagnosed with atrial myxomas by trans-thoracic echocardiography (Fig. 1, Supplementary Table S1). Two patients from the same family (siblings) were diagnosed with Carney complex. One of the siblings presented two cardiac myxomas at the time of this study with at least one previous episode documented at 6 years old (samples 1 and 7), whereas the other sibling (sample 6) previously presented cardiac myxoma whose sample was not analyzed in the present study. Before surgery, the patients developed several heart diseases as valvular obstruction, mitral disease, left heart failure or embolization. The mean age of the patients was 32 ± 15 years (range 17–53 years) consisting of 57% male and 43% female (Supplementary Table S1). Surgical excision was performed right after myxoma diagnosis to decrease the risk of valve obstruction or systemic embolization. Myxomas (0.1–1 cm width X 1–11 cm length) were mainly located in the heart left atrium (5 of 7 cases, 71%); whereas the rest were located in the left ventricle (1 of 7 cases) and right ventricle (1 of 7 cases) (Supplementary Table S1). Oncological clinic follow up indicated that none of the patients to which myxoma was removed developed metastasis to other organs until the end of this study.

For myxoma malignant biomarker profile, proteins involved in (1) epithelial–mesenchymal transition (SNAIL, fibronectin, β-catenin) (Thiery and Sleeman 2006; Nieto et al. 2016), (2) invasiveness (MMP-9 and vimentin) (Björklund and Koivunen 2005; Kidd et al. 2014); (3) oncogenicity (c-MYC, K-RAS and H-RAS) (Wang et al. 2011); (4) tumor transformation, progression and resistance (HIF-1α, p53) (Iurlaro et al. 2014) and (5) cancer metabolic adaptation (PPAR-γ) (Tachibana et al. 2008) were analyzed. Furthermore, the myxoma protein profile was compared with that of (a) healthy heart tissue derived from patients without tumor diagnosis and (b) a triple negative breast cancer (TNBC) subtype biopsies as positive control of metastatic cancer phenotype. The reasons to choose the TNBC are that it has been established that it develops one of the most aggressive and malignant phenotypes in the clinic (Haffty et al. 2006; Anders and Carey 2009) and also that its malignant biomarker profile has been widely used as a comparative parameter for cancer metastasis identification (Lehmann et al. 2011) and diagnosis (Yadav et al. 2015).

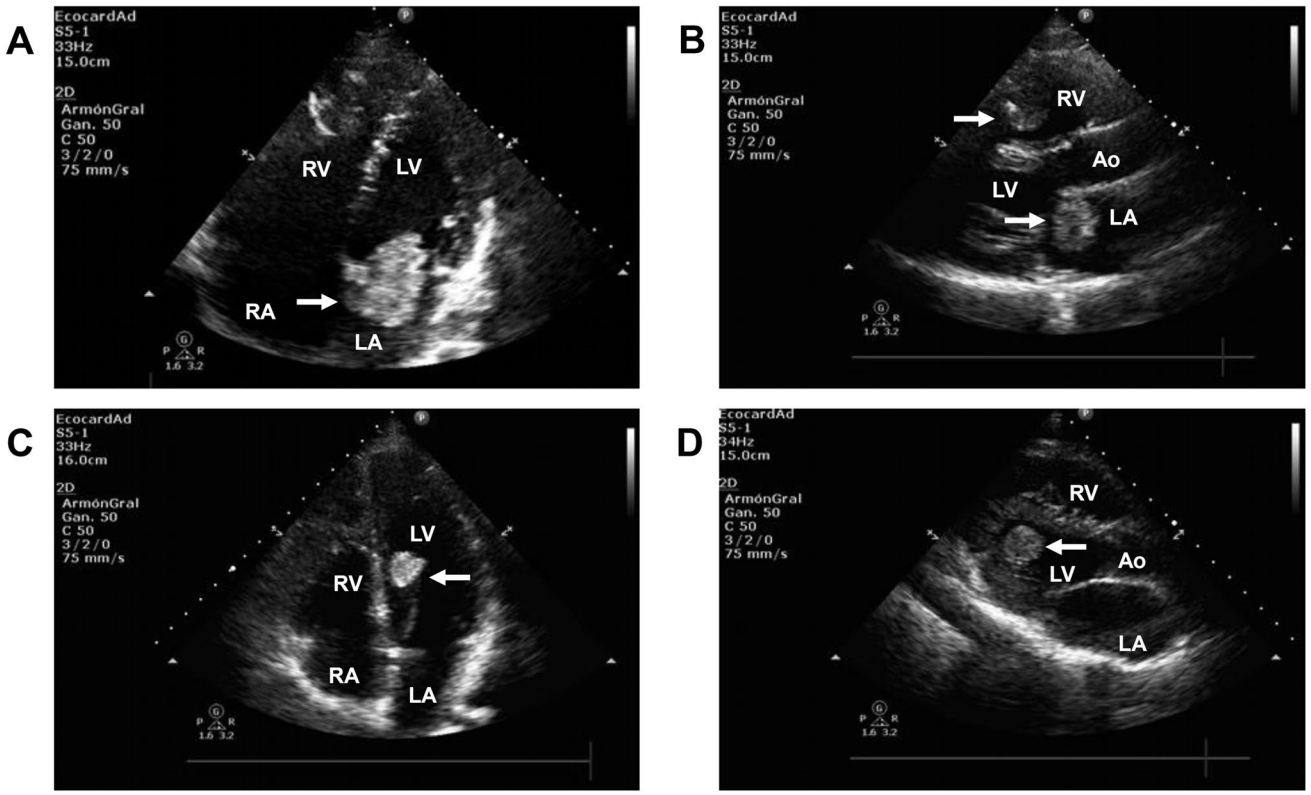


Fig. 1 Representative echocardiography of two hearts (upper and lower panels) bearing typical myxomas. **a, c** Show the four cardiac chambers and **b, d** the parasternal long axis of each heart. Myxoma is indicated with white arrows. *Ao* Aorta, *LA* left atrium, *LV* left ventricle, *RA* right atrium, *RV* right ventricle

Univariate (KW and Dunn's test) and multivariate (PCA, ROC curve) analysis for malignancy biomarker identification in heart myxomas

The content of oncogenes, transcriptional factors, and metastatic-linked proteins was determined in all tissue samples (Fig. 2a) and normalized against α-tubulin to identify their cancer phenotype profile.

Non-parametric approaches as Kruskal–Wallis and Dunn tests were used for the identification of proteins with significantly different contents in all evaluated groups (Table 1). From these analyses, both star plots and heatmap graphics were generated to facilitate the simultaneous comparison between the protein contents inside a single group, or the comparison of protein contents between different groups (Fig. 2b, c).

Multivariate approaches as PCA (principal component analysis) allowed the identification of (1) those proteins that have the highest level in all assayed groups, i.e., proteins with the highest variance value in the group and (2) those proteins with the highest variance value in the group but associated with malignancy (Fig. 3a). This information was useful to determine the patterns of distribution of the proteins between assayed groups, accordingly to the content of each protein (Fig. 3b).

Once a differential panel of proteins is identified, they were analyzed anew but using the ROC curve approach to recognize those proteins with potential biomarker use. For this, the area under the curve (AUC) and the cut-off point (i.e., the percentage of protein level vs. the percentage of α-tubulin level) for each protein was calculated. An AUC value close to 0.9–1 is indicative that protein may be considered as a good biomarker (Swets 1988) (Fig. 4; Table 2).

Content of oncogenes, metabolic cancer-associated and metastatic proteins in myxomas vs. non-cancer heart tissue

Kruskal–Wallis and Dunn's tests correction for multiple comparison (Fig. 2b, c; Table 1) analyses, multivariate (PCA, Fig. 3) and ROC (Fig. 4; Table 2) analyses revealed that the c-MYC oncogene, the p53 transcription factor, the metastatic protein vimentin and the cancer metabolic marker HIF-1α were significantly higher (85–100 fold) in myxoma biopsies compared to non-cancer heart tissue. However, only c-MYC, p53 and vimentin could be potential cancer

biomarkers because of (1) their area under ROC curves was the unit (AUC = 1); (2) their sensitivity and specificity was of 100% (Fig. 4a; Table 2) and (3) their cut-off values (30.5, 2.5 and 3.6, respectively) ensuring 0% of both false positive and/or negative results. On the contrary, HIF-1α is not an acceptable myxoma marker because its AUC is lower than unit (AUC = 0.939), its specificity was less than 100% (85%), and their cut-off value was around 60%, i.e., statistical data support that HIF-1α expression may be associated with at least 12% of false negative results (Table 2; Fig. 4a).

Although the fibronectin level was visually higher in myxomas vs. non-cancer heart tissue (Fig. 2), Kruskal–Wallis and ROC analyses showed no significant differences (Tables 1, 2). For PPAR-γ and β-catenin, the levels were very similar among all the groups (Table 1; Fig. 2).

Intriguingly, several well-known metastatic proteins such as H- and K-RAS, SNAIL, or MMP-9 whose expression is 10-fold higher in malignant cancers, breast cancer biopsies (H-and K-RAS) vs. non-cancer tissue (Pacheco-Velázquez et al. 2014; Köhrmann et al. 2009; Wu et al. 2016; Hong et al. 2017) have low expression or were absent in almost all myxoma samples. To rule out failures in all these antibodies, they were tested in MCF-7, MDA-MB-231, and MDA-MB-468 breast cancer cells. A positive signal was observed in the cancer lines assayed (Supplementary Figure S1). An interesting finding was the presence of c-MYC, a cancer progression marker (Chen and Olopade 2008) and p53 in two of the seven non-cancer heart tissue samples (Fig. 2a) suggesting that in these patients cancer development may be possible.

Content of oncogenes, metabolic cancer-associated and metastatic proteins in myxomas and its surrounding tissue

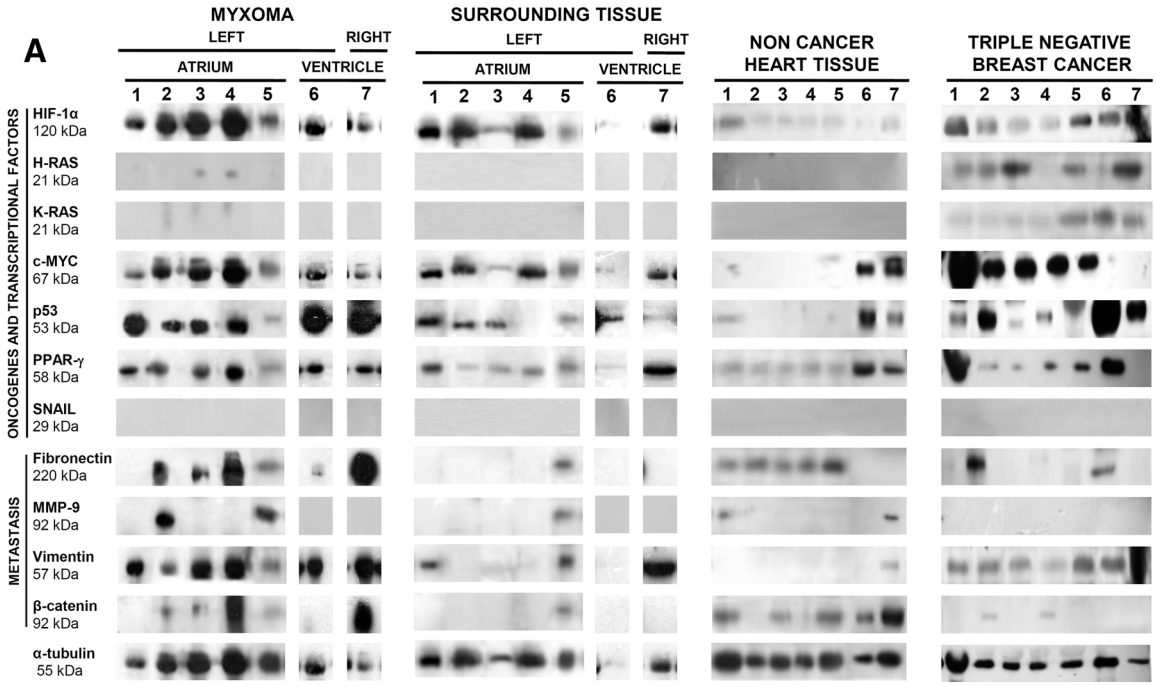
One characteristic found in heart myxomas is their high recurrence after surgery suggesting that the area close to myxoma removal may acquire a cancer phenotype. Therefore, samples from surrounding tissue were collected approximately at 4 mm from the heart zone where myxoma was removed and the proteins analyzed (Fig. 2a). The oncogenic c-MYC and the cancer metabolic biomarker HIF-1α were present in all the myxoma surrounding zones (7 of 7 samples), whereas PPAR-γ and p53 were found in 6 of 7 samples. Kruskal–Wallis test and Dunn's test correction for multiple comparison analyses revealed that the surrounding tissue is significantly different to non-cancer heart tissue; but highly similar to the oncogenic and metastatic protein pattern HIF-1α, c-MYC, and vimentin found in myxoma biopsies (Fig. 2; Table 1). Fibronectin was significantly higher (Table 1) in myxoma vs. surrounding tissue and vs. non-cancer hearth tissue. ROC analysis (AUC = 0.918) revealed that fibronectin might be considered as a potential marker for identification between myxoma and its surrounding tissue (Table 2; Fig. 4b). All these results correlate with the high recurrence of myxoma after surgical removal (Singhal et al. 2014).

Content of oncogenes, metabolic cancer-associated and metastatic proteins in myxomas compared to malignant breast carcinoma

One of the most malignant cancer types in the world is the triple negative breast cancer subtype (TNBC) (Foulkes et al. 2010; Collignon et al. 2016). To compare the myxoma vs. TNBC, the oncogenic pattern of the latter was also analyzed. All TNBC (Fig. 2) showed several over-expressed oncogenic proteins and transcriptional factors such as c-MYC, HIF-1α, H-RAS, K-RAS, p53, PPAR-γ and vimentin as expected. On the contrary, fibronectin, MMP-9 and β-catenin were remarkably absent in most of TNBC biopsies. The KW, PCA and ROC analyses revealed that TNBC and myxoma showed similar c-MYC, HIF-1α, vimentin, p53 and PPAR-γ protein contents. However, H- and K-RAS levels were significantly higher in TNBC vs. myxomas. Interestingly, the low content of MMP-9 found in TNBC was consistent with the decreased level found in 5 of 7 myxoma samples. Regarding β-catenin, its content was remarkably different between TNBC (null expression) and myxoma (significantly high vs. TNBC) (Fig. 2; Table 1). The overall analysis of metastatic proteins found in TNBC vs. myxoma suggests that myxoma over-express the typical TNBC oncogenic pattern (p values > 0.05, Table 1).

Identification of oncogenic biomarkers in atrial myxoma

The univariate (KW and star plots, Fig. 2b; Table 1) and multivariate (ROC and PCA, Figs. 3, 4; Table 2) analyses between myxoma and non-cancer heart samples revealed significant differences in the contents of some oncogenes, transcription factors, and metastatic proteins. The PCA analysis (Fig. 3a) provided a differentiated and selective panel of cancer biomarkers constituted by **c-MYC = p53 = vimentin > HIF-1α** for atrial myxoma vs. non-cancer heart tissue; whereas the same proteins did not show significant differences in myxoma vs. surrounding tissue or TNBC. The rest of the proteins assayed (H-RAS, K-RAS, MMP-9, β-catenin) have been identified as malignant markers in several cancer cells (Lin et al. 2000; Kalia 2015) even all these proteins where highly expressed in TNBC (Pacheco-Velázquez et al. 2014); however, KW and ROC analyses revealed non-significant changes between myxomas and non-cancer heart tissue (Tables 1, 2). Thus these proteins would not be useful for malignity prognostic.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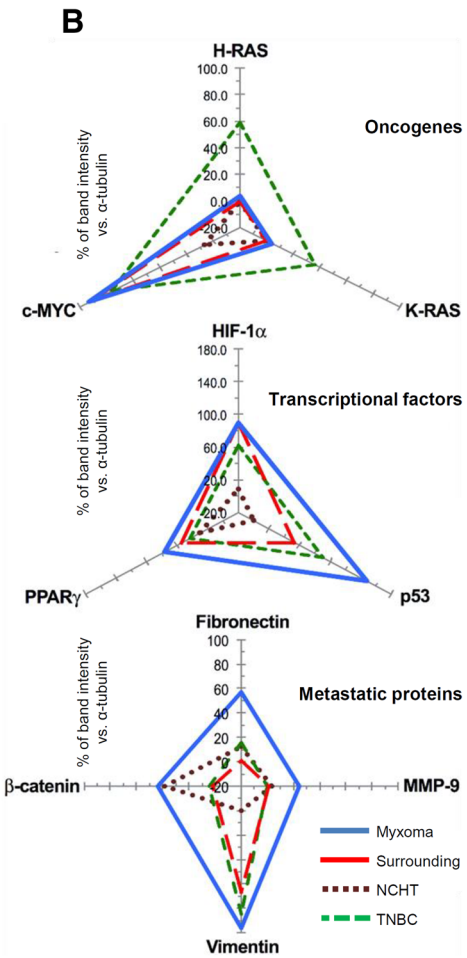
H-RAS 21 kDa
K-RAS 21 kDa
c-MYC 67 kDa
p53 53 kDa
PPAR-γ 58 kDa
SNAIL 29 kDa
Fibronectin 220 kDa
MMP-9 92 kDa
Vimentin 57 kDa
β-catenin 92 kDa
α-tubulin 55 kDa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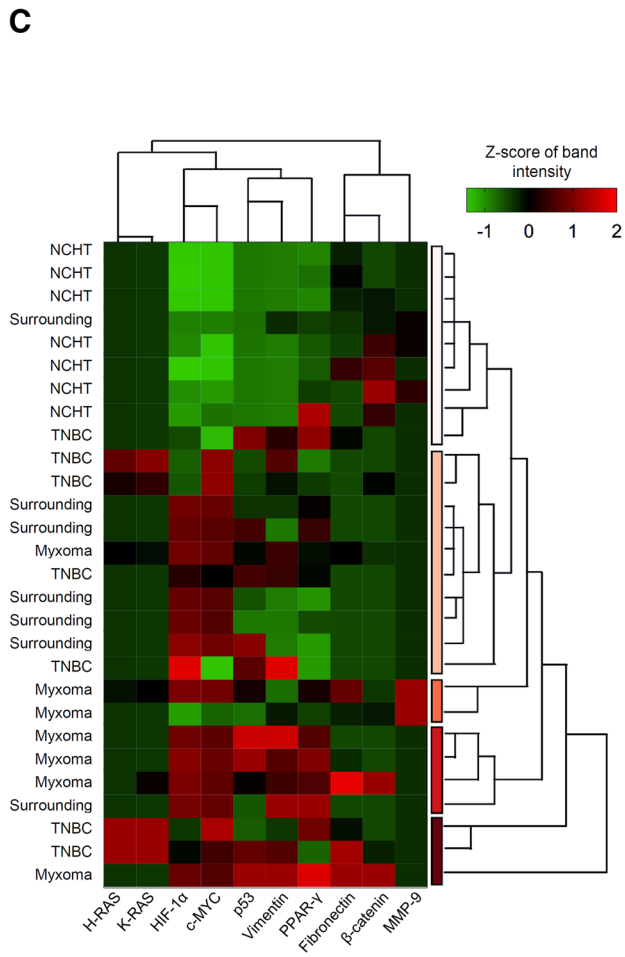
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Fibronectin
β-catenin
MMP-9

◂**Fig. 2** Oncogene, metastatic, and metabolic proteins expression in heart myxoma, surrounding cardiac tissue, non-cancer heart tissue (NCHT) and triple negative breast cancer (TNBC). **a** Representative western blots showing analyzed protein contents; **b** star plots and **c** Heat map of *Z* scores and dendrogram analysis. Lower or higher proteins are indicated with green or red color, respectively. Assayed proteins of each group were compared with their respective load control α-tubulin in each analysis. $n=7$ for all biopsy samples. *HIF-1α* hypoxia-inducible factor 1 alpha, *H-RAS* Harvey rat sarcoma viral oncogene homolog, *K-RAS* Kirsten rat sarcoma viral oncogene homolog, *c-MYC* avian myelocytomatosis viral oncogene homolog, *PPAR-γ* peroxisome proliferator-activated receptor gamma, *SNAIL* zinc finger protein SNAI1, *MMP-9* matrix metallopeptidase 9

Discussion

Myxomas have been classified as the most common benign primary heart tumors with a high rate of recurrence (Reynen 1996; Hoffmeier et al. 2014). However, the molecular mechanisms underlying neoplasic transformation from heart normal cells to myxoma remain unclear. Because of its apparent benign nature, there are not oncologic medical treatments proposed against heart myxoma (Hoffmeier et al. 2014); thus, once it recurs, surgery is newly programmed and executed with all the risks that it involves.

Recurrence of heart myxoma can be attributable to malignant transformation

After surgical resection, the frequency in unprompted myxomas recurrence is < 5%, however, this frequency increases (> 20%) in familial-type myxomas (Becker et al. 2008; Singhal et al. 2014; Villalpando-Mendoza et al. 2006). Without anti-oncologic treatment, myxoma recurrence is becoming more common, which has been attributed to: (1) incomplete tumor excision (Mendoza et al. 2007); (2) intracardiac implantation from the primary tumor; or (3) malignant transformation (Becker et al. 2008; Pinede et al. 2001). Although cardiac myxomas are usually considered as benign-type tumors, it has been demonstrated that a subpopulation develops an intrinsic malignant potential including: (1) more aggressive patterns of recurrence (Mendoza et al. 2007); (2) similar histological changes to that observed in malignant heart sarcomas (Singhal et al. 2014; Hasegawa et al. 2002); (3) abnormal DNA ploidy pattern (McCarthy et al. 1989), and (4) in some cases distant metastases (Wada et al. 1993; Hou et al. 2001). Additionally, it has been suggested that the multiple recurrences of myxomas correspond to a multifocal disease (Mendoza et al. 2007) and several others factors including dissemination of neoplastic cells after surgery in embolic fragments (Singhal et al. 2014).

Myxoma and surrounding myxoma tissue show similarity with the malignant protein profile found in malignant cancers

There is scarce literature showing a cancer biomarker profile in hearth myxoma for its better diagnosis and treatment. Malignant cancers as breast, glioma, prostate, colorectal, and lung carcinomas over-express specific biomarker proteins of malignancy and metastasis properties (Locker et al. 2006; Henry and Hayes 2012; Goossens et al. 2015). Notably, in triple negative breast cancer (TNBC), oncogenes (c-MYC, H-RAS, K-RAS, p53), proteins related with

Table 1 Median (Interquartile range) values of protein expression according to the percentage of band intensity with respect to α-tubulin in myxoma, surrounding tissue, non-cancer heart tissue (NCHT) and triple negative breast cancer (TNBC)

Function	Protein	Group ($n=7$)			
		Myxoma	Surrounding	NCHT	TNBC
Oncogenes	H-RAS	0 (0–5)	0 (0–0)[d]	0 (0–0)[e]	23 (0-109)
	K-RAS	0 (0–9)	0 (0–0)[d]	0 (0–0)[e]	20 (0–69)
	c-MYC	102 (33–104)[b]	99 (24–105)[c]	0 (0–7)[e]	91 (0-119)
Transcriptional factors	HIF-1α	101 (14–105)[b]	99 (23–102)[c]	1 (0–17)[e]	46 (34–68)
	p53	87 (4-256)[b]	22 (0–82)	0 (0–0)[e]	118 (16–139)
	PPAR-γ	81 (30–91)	30 (0–63)	21 (7–26)	32 (0–70)
Metastasis markers	Fibronectin	22 (0–77)[a,b]	0 (0–0)	12 (0–16)	0 (0–18)
	MMP-9	0 (0–36)	0 (0–0)	0 (0–5)	0 (0–0)
	Vimentin	85 (7-127)[b]	2 (0–34)	0 (0–0)[e]	82 (31–96)
	β-catenin	7 (0–45)	0 (0–0)	40 (0–47)	0 (0–6)

Groups were compared by means of non-parametric Kruskal–Wallis test was used followed by Dunn's test protected for multiple comparisons (Dunn 1964) using $p<0.05$ as statically significant for the family of comparisons. Differences between groups are indicated as [a]myxoma vs. surrounding, [b]myxoma vs. NCHT, [c]surrounding tissue vs. NCHT, [d]surrounding tissue vs. TNBC, and [e]between NCHT vs. TNBC. No differences were found between myxoma vs. TNBC

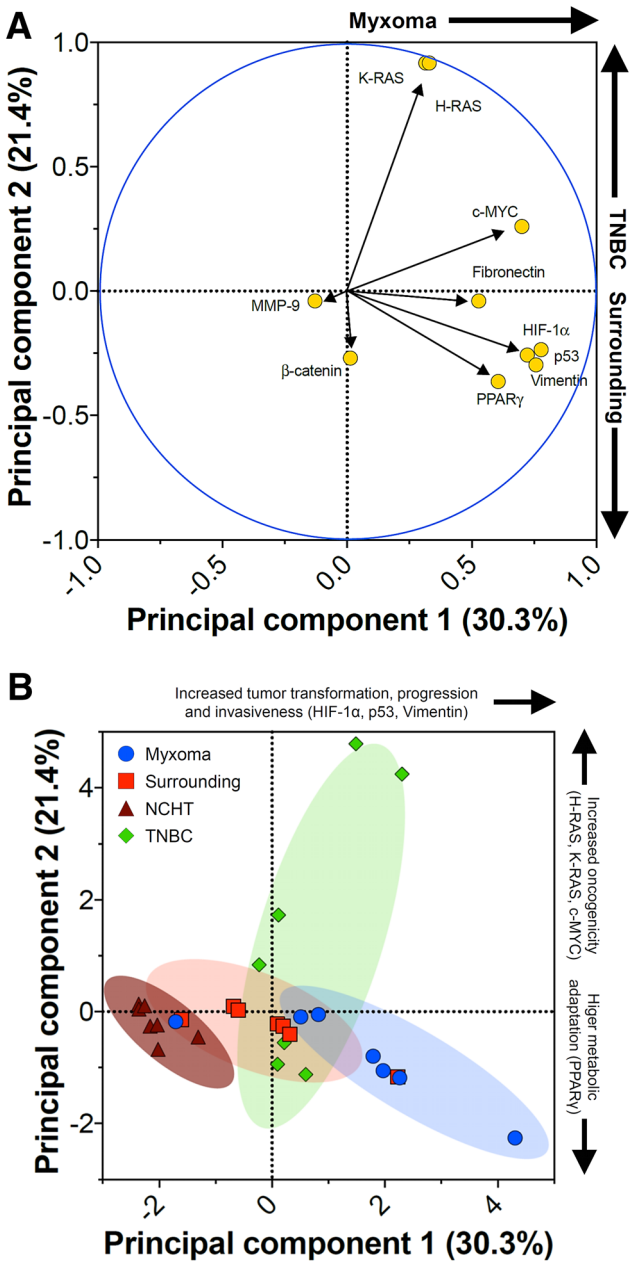

Fig. 3 Patterns of association of behavior of protein expression in myxoma, surrounding cardiac tissue, non-cancer heart tissue and triple negative breast cancer. **a** Evaluated outcomes loadings for each protein form the two first principal components, which in conjunction explained 51.7% of the total variation, and **b** individual scores for each myxoma, surrounding tissue, non-cancer heart tissue (NCHT) and triple negative breast cancer (TNBC) according to principal component axes 1 and 2. Also note that the main factors that separated myxoma and NCHT were an increased expression of proteins involved in tumor transformation, progression, and invasiveness, as well as higher metabolic adaptation. In contrast, an increased oncogenicity separates the TNBC group from the rest. Arbitrary ellipses were drawn to assist interpretation of the pattern of association among groups. Abbreviations as in Fig. 2

specific metabolic and growth functions (COX-2, 2OGDH, E-cadherin, EGFR, VEGF) have been identified as reliable biomarkers for cancer detection (Pacheco-Velázquez et al. 2014; Yadav et al. 2015; Wang et al. 2016). In this study, analysis of heart myxoma biopsies showed a protein biomarker pattern of **c-MYC = p53 = vimentin > HIF-1α** which was very close to that found in well-characterized malignant TNBC **HIF-1α = p53 = vimentin > c-MYC**. This finding strongly suggests that myxomas have the potential to developing a malignant phenotype and they should be considered as malignant tumors.

Although HIF-1α has been considered as a cancer biomarker in solid tumors (Talks et al. 2000; Zhong et al. 1999), this protein can be stabilized under ischemic events in heart (Goswami and Das 2010; Loor and Schumacker 2008) or in other illnesses such as atherosclerosis, inflammatory disorders, and pre-eclampsia (Gao et al. 2012; Tal 2012; Biddlestone et al. 2015). Thus, the observed changes in the content of this protein in myxomas may not necessarily be associated with cancer development. To circumvent this matter, HIF-1α protein level should be analyzed in parallel to well-known oncogenic markers (Rodríguez-Enríquez et al. 2011).

Interestingly, the oncogenic pattern found in myxomas was also present in the surrounding tissue, suggesting that surgery may not be sufficient to remove all cancer tissue. In this regard, It has been demonstrated that several tumor cells (breast, lung, prostate carcinomas) may migrate to the adjacent lymph nodes (i.e., showing micrometastasis) for de novo tumor generation (Mohajeri et al. 2012; Porcaro et al. 2017). There is scarce information regarding possible molecular mechanisms involved in cellular spreading and metastasis activation in heart myxomas thus far. Several tumors as colorectal and neck carcinomas develop filiform projections that correlate with their infiltrative capacity to impels metastasis (Tomifuji et al. 2011; Kim et al. 2016). Although, there are not studies indicating that myxomas develop these projections or have infiltrative potential, both of them may be considered as potential markers of metastasis progression.

Several studies have been focused in the recognition of malignant proteins in various benign heart tumors. In papillary fibroelastomas and dedifferentiated hearth cells, overexpression of the malignant oncogenes K-RAS (mutated isoform) and c-MYC, respectively (Wittersheim et al. 2017; Jackson et al. 1990) have been found. Additionally, it has been demonstrated that myxomas produce numerous growth factors and cytokines, including vascular endothelial growth factor, resulting in angiogenesis and tumor growth and increased expression of interleukin-6 (Wada et al. 1993). Additionally, metastatic proteins such as PDGF A-B,

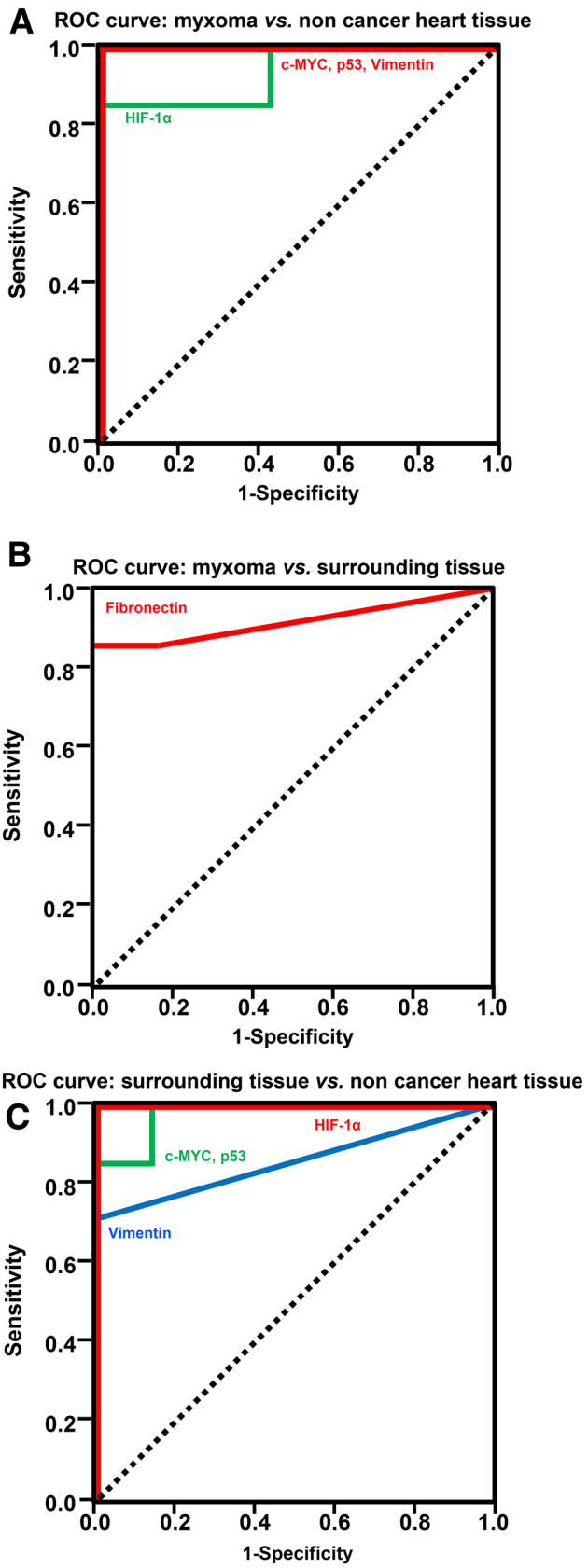

Fig. 4 Identification of biomarker proteins in myxoma. ROC analysis shows the performance of several proteins significantly different between **a** myxomas vs. non-cancer heart tissue, **b** myxomas vs. surrounding cardiac tissue and **c** surrounding cardiac tissue vs. non-cancer heart tissue. Abbreviations as in Fig. 2 ▶

PDGFR, TYMP, VEGF-A, VEGFR 1–2, MMPs (1–3, 9 and 14), vimentin, IL-6, FGFβ, FGFR (Singhal et al. 2014; Wada et al. 1993; Barh et al. 2009). are found in heart myxoma, reinforcing the proposal that myxoma develops a malignant phenotype (Singh et al. 2015; Villalpando-Mendoza et al. 2006).

Prognostic value of the expression of malignant protein biomarkers in hearth myxomas: Clinical implication

Myxomas are not considered a health issue problem because (a) their prevalence is approximately 0.02–0.05% (200–500 tumors per million autopsies) (Becker et al. 2008; Isobe and Murohara 2015); (b) sudden death only occurs in 15% of the patients with atrial myxoma; and (c) > 95% of the patients have a survival prognosis of 10 years. However, these epidemiological analyses have not considered myxomas recurrence, in which a metastatic phenotype progresses without specific oncologic treatment. Furthermore, several recurrent myxomas have been associated with distant metastasis in brain (Hou et al. 2001; Altundag et al. 2005); adrenal glands (Kaul et al. 2013) and superior limbs and inguinal region (Kaynak et al. 2001). In this last case, malignancy of myxomas was identified solely by histological examination. Therefore, it is necessary the implementation of specific heart myxoma malignancy biomarkers.

In conclusion, this pilot study reveals that all analyzed heart myxoma samples exhibited high levels of oncogene, metastatic, and tumor-associated metabolic proteins. Therefore, heart myxoma should not be considered as a benign tumor and once heart myxoma is surgically removed its oncogenic pattern should be determined as potential of malignancy. Further prospective studies should still be performed to associate these findings with local or global tumor progression. However, the results derived from this study may help clinicians to define the adequate chemotherapy treatment schemes to avoid myxoma recurrence. For example, if the oncogenic pattern is positive, then chemotherapy treatment directed to TNBC tamoxifen, 5-fluorouracil, doxorubicin, cyclophosphamide, trastuzumab (Cardoso et al. 2012) could be applied to post-operated patients as an alternative therapeutic strategy against recurrent myxoma.

Table 2 ROC parameters, cut-off points, sensitivity and specificity percentages of different biomarkers found in myxoma

Protein	AUC	*P* value ROC Curve	Cut-off value	Sensitivity %	Specificity %	PPV %	NPV %
ROC curve analysis: myxomas vs. non-cancer heart tissue							
HIF-1α	0.939	0.006	7.5	100	57.1	63	100
			20	85.7	85.7	86	86
			59.5	85.7	100	100	88
c-MYC	1.00	0.002	7.5	100	71.4	78	100
			21.5	100	85.7	88	100
			30.5	100	100	100	100
p53	1.00	0.002	0.5	100	85.7	88	100
			2.5	100	100	100	100
			3.6	85.7	100	100	100
Vimentin	1.00	0.002	3.6	100	100	100	100
			25.6	85.7	100	100	88
ROC Curve analysis: myxomas vs. surrounding tissue							
Fibronectin	0.918	0.009	3	85.7	85.7	86	86
			7.45	85.7	100	100	88
ROC Curve analysis: surrounding tissue vs. non-cancer heart tissue							
HIF-1α	1.00	0.002	0.5	100	28.6	58	100
			17	100	71.4	78	100
			22	100	100	100	100
c-MYC	0.98	0.003	7.5	100	71.4	78	100
			26	85.7	85.7	86	86
			62.5	85.7	100	100	88
P53	0.98	0.003	0.1	100	85.7	88	100
			0.6	85.7	85.7	86	86
			2	85.7	100	100	88
Vimentin	0.857	0.025	0.75	71.4	100	100	78
			1.8	57.1	100	100	70

AUC area under curve, *ROC* receiver operative characteristic, *PPV* positive predictive value, *NPV* negative predictive value

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval All procedures involving human samples (myxomas, surrounding tissue, non-cancer heart tissue, and breast cancer tissue) were performed in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

Abba MC, Lacunza E, Butti M, Aldaz CM (2010) Breast cancer biomarker discovery in the functional genomic age: a systematic review of 42 gene expression signatures. Biomark Insights 5:103–118. https://doi.org/10.4137/BMI.S5740

Aguirre-Benítez EL, Porras MG, Parra L, González-Ríos J, Garduño-Torres DF, Albores-García D, Avendaño A, Ávila-Rodríguez MA, Melo AI, Jiménez-Estrada I, Mendoza-Garrido ME, Toriz C, Diaz D, Ibarra-Coronado E, Mendoza-Ángeles K, Hernández-Falcón J (2017) Disruption of behavior and brain metabolism in artificially reared rats. Dev Neurobiol 77(12):1413–1429. https://doi.org/10.1002/dneu.22548

Altundag MB, Ertas G, Ucer AR, Durmus S, Abanuz H, Calikoğlu T, Ozbagi K, Demirkasimoglu A, Kaya B, Bakkal BH, Altundag K (2005) Brain metastasis of cardiac myxoma: case report and review of the literature. J Neurooncol 75(2):181–184. https://doi.org/10.1007/s11060-005-1859-7

Anders CK, Carey LA (2009) Biology, metastatic patterns, and treatment of patients with triple-negative breast cancer. Clin Breast Cancer 9(Suppl 2):S73–S81. https://doi.org/10.3816/CBC.2009.s.008

Barh D, Kumar A, Chatterjee S, Liloglou T (2009) Molecular features, markers, drug targets, and prospective targeted therapeutics in cardiac myxoma. Curr Cancer Drug Targets 9(6):705–716. https://doi.org/10.2174/156800909789271549

Bartlett JEII, Kotrlik JW, Higginsm CC (2001) Organizational research: determining appropriate sample size in survey. Off Syst Res J 19(1):43–50. https://doi.org/10.5306/wjco.v6.i6.252

Becker RP, Ramírez MA, Zalaquett SR, Moran VS, Irarrázaval LI MJ, Arretz VC, Córdova AS, Arnaiz GP (2008) Cardiac myxoma: clinical characterization, diagnostic methods and late surgical results. Rev Med Chil 136(3):287–295

Biddlestone J, Bandarra D, Rocha S (2015) The role of hypoxia in inflammatory disease (review). Int J Mol Med 35(4):859–869. https://doi.org/10.3892/ijmm.2015.2079

Björklund M, Koivunen E (2005) Gelatinase-mediated migration and invasion of cancer cells. Biochim Biophys Acta 1755(1):37–69. https://doi.org/10.1016/j.bbcan.2005.03.001

Blackmon SH, Kassis ES, Ge Y, Goldfarb R, Reardon M (2010) Left atrial myxoma embolus to the renal artery: should a nephrectomy be advised? Ann Thorac Surg 90(1):289–292. https://doi.org/10.1016/j.athoracsur.2009.11.059

Burke A, Jeudy J, Virmani R (2007) Textbook of cardiovascular medicine. Lippincott Williams and Wilkins, Philadelphia

Cardoso F, Harbeck N, Fallowfield L, Kyriakides S, Senkus E, ESMO Guidelines Working Group (2012) Locally recurrent or metastatic breast cancer: ESMO clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 23(Suppl 7):vii11–9. https://doi.org/10.1093/annonc/mds232

Carney JA (1985) Differences between non-familial and familial cardiac myxoma. Am J Surg Pathol 9(1):53–55. https://doi.org/10.1097/00000478-198501000-00009

Chambers JM, Cleveland WS, Kleiner B, Tukey PA (1983) Graphical methods for data analysis. Chapman and Hall/Cole Publishing Company, London

Chen Y, Olopade OI (2008) MYC in breast tumor progression. Expert Rev Anticancer Ther 8(10):1689–1698. https://doi.org/10.1586/14737140.8.10.1689

Collignon J, Lousberg L, Schroeder H, Jerusalem G (2016) Triple-negative breast cancer: treatment challenges and solutions. Breast Cancer (Dove Med Press) 8:93–107. https://doi.org/10.2147/BCTT.S69488

Diaz D, Vazquez-Polanco AM, Argueta-Donohué J, Stephens CR, Jimenez-Trejo F, Ceballos-Liceaga SE, Mantilla-Beniers N (2018) Incidence of intestinal infectious disease due to protozoa and bacteria in Mexico: analysis of national surveillance records from 2003 to 2012. BioMed Res Int 2018:22. https://doi.org/10.1155/2018/2893012

Dunn OJ (1964) Multiple comparisons using rank sums. Technometrics 6:241–252. https://doi.org/10.1080/00401706.1964.10490181

Foulkes WD, Smith IE, Reis-Filho JS (2010) Triple-negative breast cancer. N Engl J Med 363(20):1938–1948. https://doi.org/10.1056/NEJMra1001389

Fyke FE 3rd, Seqard JB, Edwards WD, Miller FA Jr, Reeder GS, Schattenberg TT, Schub C, Callahan JA, Tajik AJ (1985) Primary cardiac tumors: experience with 30 consecutive patients since the introduction of two-dimensional echocardiography. J Am Coll Cardiol 5(6):1465–1473. https://doi.org/10.1016/S0735-1097(85)80364-8

Gallardo-Pérez JC, Rivero-Segura NA, Marín-Hernández A, Moreno-Sánchez R, Rodríguez-Enríquez S (2014) GPI/AMF inhibition blocks the development of the metastatic phenotype of mature multi-cellular tumor spheroids. Biochim Biophys Acta 1843(6):1043–1053. https://doi.org/10.1016/j.bbamcr.2014.01.013

Gao L, Chen Q, Zhou X, Fan L (2012) The role of hypoxia-inducible factor 1 in atherosclerosis. J Clin Pathol 65(10):872–876. https://doi.org/10.1136/jclinpath-2012-200828

Goossens N, Nakagawa S, Sun X, Hoshida Y (2015) Cancer biomarker discovery and validation. Transl Cancer Res 4(3):256–269. https://doi.org/10.3978/j.issn.2218-676X.2015.06.04

Gošev I, Paić F, Durić Z, Gošev M, Ivčević S, Jakuš FB, Biočina B (2013) Cardiac myxoma the great imitators: comprehensive histopathological and molecular approach. Int J Cardiol 164(1):7–20. https://doi.org/10.1016/j.ijcard.2011.12.052

Goswami SK, Das DK (2010) Oxygen sensing, cardiac ischemia, HIF-1α and some emerging concepts. Curr Cardiol Rev 6(4):265–273. https://doi.org/10.2174/157340310793566136

Haffty BG, Yang Q, Reiss M, Kearney T, Higgins SA, Weidhaas J, Harris L, Hait W, Toppmeyer D (2006) Locoregional relapse and distant metastasis in conservatively managed triple negative early-stage breast cancer. J Clin Oncol 24(36):5652–5657. https://doi.org/10.1200/JCO.2006.06.5664

Hannah H 3rd, Eisemann G, Hiszcznskyj R, Winsky M, Cohen L (1982) Invasive atrial myxoma: documentation of malignant potential of cardiac myxomas. Am Heart J 104(4 Pt 1):881–883. https://doi.org/10.1016/0002-8703(82)90031-X

Hasegawa T, Nakagawa S, Chino M, Kunihiro T, Ui S, Kimura M (2002) Primary cardiac sarcoma mimicking benign myxoma: a case report. J Cardiol 39(6):321–325

Henry NL, Hayes DF (2012) Cancer biomarkers. Mol Oncol 6(2):140–146. https://doi.org/10.1016/j.molonc.2012.01.010

Hoffmeier A, Sindermann JR, Scheld HH, Martens S (2014) Cardiac tumors—diagnosis and surgical treatment. Dtsch Arztebl Int 111(12):205–211. https://doi.org/10.3238/arztebl.2014.0205

Hong OY, Noh EM, Jang HY, Lee YR, Lee BK, Jung SH, Kim JS, Youn HJ (2017) Epigallocatechin gallate inhibits the growth of MDA-MB-231 breast cancer cells via inactivation of the β-catenin signaling pathway. Oncol Lett 14(1):441–446. https://doi.org/10.3892/ol.2017.6108

Hou YC, Chang S, Lo HM, Hsiao CH, Lin FY (2001) Recurrent cardiac myxoma with multiple distant metastasis and malignant change. J Formos Med Assoc 100(1):63–65

Ibarra-Coronado EG, Velazquéz-Moctezuma J, Diaz D, Becerril-Villanueva LE, Pavón L, Morales-Montor J (2015) Sleep deprivation induces changes in immunity in Trichinella spiralis-infected rats. Int J Biol Sci 11(8):901–912. https://doi.org/10.7150/ijbs.11907

Isobe S, Murohara T (2015) Editorial: cardiac tumors: histopathological aspects and assessments with cardiac noninvasive imaging. J Cardiol Cases 12:37–38. https://doi.org/10.1016/j.jccase.2015.04.008

Iurlaro R, León-Annicchiarico CL, Muñoz-Pinedo C (2014) Regulation of cancer metabolism by oncogenes and tumor suppressors. Methods Enzymol 542:59–80. https://doi.org/10.1016/B978-0-12-416618-9.00003-0

Jackson T, Allard MF, Sreenan CM, Doss LK, Bishop SP, Swain JL (1990) The c-myc proto-oncogene regulates cardiac development in transgenic mice. Mol Cell Biol 10(7):3709–3716. https://doi.org/10.1128/MCB.10.7.3709

Kalia M (2015) Biomarkers for personalized oncology: recent advances and future challenges. Metabolism 64(3 Suppl 1):S16–S21. https://doi.org/10.1016/j.metabol.2014.10.027

Kaul P, Paniagua R, Balaji S, Batin P (2013) Large mass in right atrium after CABG—myxoma, adrenal metastasis or in-transit thrombus? Br J Cardiol 20:79. https://doi.org/10.5837/bjc.2013.19

Kaynak K, Beşirli K, Arslan C, Ozgüroğlu M, Oz B (2001) Metastatic cardiac myxoma. Ann Thorac Surg 72(2):623–625. https://doi.org/10.1016/S0003-4975(00)02408-5

Kidd ME, Shumaker DK, Ridge KM (2014) The role of vimentin intermediate filaments in the progression of lung cancer. Am J Respir Cell Mol Biol 50(1):1–6. https://doi.org/10.1165/rcmb.2013-0314TR

Kim SA, Inamura K, Yamauchi M, Nishihara R, Mima K, Sukawa Y, Li T, Yasunari M, Morikawa T, Fitzgerald KC, Fuchs CS, Wu K, Chan AT, Zhang X, Ogino S, Qian ZR (2016) Loss of CDH1 (E-cadherin) expression is associated with infiltrative tumour growth and lymph node metastasis. Br J Cancer 114(2):199–206. https://doi.org/10.1038/bjc.2015.347

Köhrmann A, Kammerer U, Kapp M, Dietl J, Anacker J (2009) Expression of matrix metalloproteinases (MMPs) in primary human breast cancer and breast cancer cell lines: New findings and review of the literature. BMC Cancer 9:188. https://doi.org/10.1186/1471-2407-9-188

Kothari J, Lakhia K, Solanki P, Panchal J, Shah P (2016) Recurrent left atrial myxoma in a young patient: a rare entity. J Clin Diagn Res 10(8):PD05–P7. https://doi.org/10.7860/JCDR/2016/20425.8321

Lee VH, Connolly HM, Brown RD Jr (2007) Central nervous system manifestations of cardiac myxoma. Arch Neurol 64(8):1115–1120. https://doi.org/10.1001/archneur.64.8.1115

Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, Pietenpol JA (2011) Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 121(7):2750–2767. https://doi.org/10.1172/JCI45014

Lin SY, Xia W, Wang JC, Kwong KY, Spohn B, Wen Y, Pestell RG, Hung MC (2000) Beta-catenin, a novel prognostic marker for breast cancer: its roles in cyclin D1 expression and cancer progression. Proc Natl Acad Sci USA 97(8):4262–4266. https://doi.org/10.1073/pnas.060025397

Locker GY, Hamilton S, Harris J, Jessup JM, Kemeny N, Macdonald JS, Somerfield MR, Hayes DF, Bast RC Jr, ASCO (2006) ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. J Clin Oncol 24(33):5313–5327. https://doi.org/10.1200/JCO.2006.08.2644

Loor G, Schumacker PT (2008) Role of hypoxia-inducible factor in cell survival during myocardial ischemia-reperfusion. Cell Death Differ 15(4):686–690. https://doi.org/10.1038/cdd.2008.13

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193(1):265–275

McCarthy PM, Schaff HV, Winkler HZ, Lieber MM, Carney JA (1989) Deoxyribonucleic acid ploidy pattern of cardiac myxomas. Another predictor of biologically unusual myxomas. J Thorac Cardiovasc Surg 98(6):1083–1086

Mendoza C, Bernstein E, Ferreira A (2007) Multiple recurrences of nonfamilial cardiac myxomas: a report of two cases. Tex Heart Inst J 34(2):236–239

Mohajeri G, Sanei MH, Tabatabaee SA, Hashemi SM, Amjad E, Mohajeri MR, Shemshaki H, Jazi AH, Kolahdouzan M (2012) Micrometastasis in non-small-cell lung cancer: detection and staging. Ann Thorac Med 7(3):149–152. https://doi.org/10.4103/1817-1737.98848

Nieto MA, Huang RY, Jackson RA, Thiery JP (2016) EMT: 2016. Cell 166:21–45. https://doi.org/10.1016/j.cell.2016.06.028

Pacheco-Velázquez SC, Gallardo-Pérez JC, Aguilar-Ponce JL, Villarreal P, Ruiz-Godoy L, Pérez-Sánchez M, Marín-Hernández A, Ruiz-García E, Meneses-García A, Moreno-Sánchez R, Rodríguez-Enríquez S (2014) Identification of a metabolic and canonical biomarker signature in Mexican HR+/HER2-, triple positive and triple-negative breast cancer patients. Int J Oncol 45(6):2549–2559. https://doi.org/10.3892/ijo.2014.2676

Pateras IS, Evangelou K, Tsimaratou K, Liontos M, Sakellariou S, Barlogiannis T, Karakitsos P, Papalois A, Kotsinas A, Gorgoulis VG (2012) Detection of herplex simplex virus-1 and -2 in cardiac myxomas. J Biomed Biotechnol 2012:823949. https://doi.org/10.1155/2012/823949

Pérez-Andreu J, Parrilla G, Arribas JM, García-Villalba B, Lucas JJ, Garci- Navarro M, Marín F, Gutierrez F, Moreno A (2013) Neurological manifestations of cardiac myxoma: experience in a referral hospital. Neurologia 28(9):529–534. https://doi.org/10.1016/j.nrl.2013.03.006

Pinede L, Duhaut P, Loire R (2001) Clinical presentation of left atrial cardiac myxoma. A series of 112 consecutive cases. Medicine (Baltimore) 80(3):159–172

Porcaro AB, de Luyk N, Corsi P, Sebben M, Tafuri A, Tamanini I, Processali T, Cerruto MA, Migliorini F, Brunelli M, Siracusano S, Artibani W (2017) Bilateral lymph node micrometastases and seminal vesicle invasion associated with same clinical predictors in localized prostate cancer. Tumori 103(3):299–306. https://doi.org/10.5301/tj.5000546

Rathore KS, Hussenbocus S, Stuklis R, Edwards J (2008) Novel strategies for recurrent cardiac myxoma. Ann Thorac Surg 85(6):2125–2126. https://doi.org/10.1016/j.athoracsur.2007.11.080

Reynen K (1995) Cardiac myxomas. N Engl J Med 333(24):1610–1617. https://doi.org/10.1056/NEJM199512143332407

Reynen K (1996) Frequency of primary tumors of the heart. Am J Cardiol 77(1):107. https://doi.org/10.1016/S0002-9149(97)89149-7

Rodríguez-Enríquez S, Pacheco-Velázquez SC, Gallardo-Pérez JC, Marín-Hernández A, Aguilar-Ponce JL, Ruiz-García E, Ruizgodoy-Rivera LM, Meneses-García A, Moreno-Sánchez R (2011) Multi-biomarker pattern for tumor identification and prognosis. J Cell Biochem 112(10):2703–2715. https://doi.org/10.1002/jcb.23224

Roldán FJ, Vargas-Barrón J, Espinola-Zavaleta N, Keirns C, Romero-Cárdenas A (2000) Recurrent myxoma implanted in the left atrial appendage. Echocardiography 17(2):169–171. https://doi.org/10.1111/j.1540-8175.2000.tb01119.x

Siltanen P, Tuuteri L, Norio R, Tala P, Ahrenberg P, Halonen PI (1976) Atrial myxoma in a family. Am J Cardiol 38(2):252–256. https://doi.org/10.1016/0002-9149(76)90158-2

Singh S, Singh M, Kovacs D, Benatar D, Khosla S, Singh H (2015) A rare case of an intracardiac lipoma. Int J Surg Case Rep 9:105–108. https://doi.org/10.1016/j.ijscr.2015.02.024

Singhal P, Luk A, Rao V, Butany J (2014) Molecular basis of cardiac myxomas. Int J Mol Sci 15(1):1315–1337. https://doi.org/10.3390/ijms15011315

Smith M, Chaudhry MA, Lozano P, Humphrey MB (2012) Cardiac myxoma induced paraneoplastic syndromes: a review of the literature. Eur J Intern Med 23(8):669–673. https://doi.org/10.1016/j.ejim.2012.05.015

Suvarna SK, Royds JA (1996) The nature of the cardiac myxoma. Int J Cardiol 57(3):211–216. https://doi.org/10.1016/S0167-5273(96)02827-6

Swets JA (1988) Measuring the accuracy of diagnostic systems. Science 240(4857):1285–1293. https://doi.org/10.1126/science.3287615

Tachibana K, Yamasaki D, Ishimoto K, Doi T (2008) The role of PPARs in cancer. PPAR Res 2008:102737, https://doi.org/10.1155/2008/102737

Tal R (2012) The role of hypoxia and hypoxia-inducible factor-1alpha in preeclampsia pathogenesis. Biol Reprod 87(6):134. https://doi.org/10.1095/biolreprod.112.102723

Talks K, Turley H, Gatter KC (2000) The expression and distribution of hypoxia inducible factors HIF-1a and HIF-2a in normal human tissues, cancers tumor associated macrophages. Am J Pathol 157(2):411–421. https://doi.org/10.1016/S0002-9440(10)64554-3

Tarelo-Saucedo JM, Peñaloza-Guadarrama M, Villela-Caleti J, García-Cruz A, Arizmendi-Monroy DK, Reynada-Torres JL, Martinez-Ramirez L (2016) Surgical results and monitoring of postoperative atrial myxomas. Arch Cardiol Mex 86(1):35–40. https://doi.org/10.1016/j.acmx.2015.09.002

Thiery JP, Sleeman JP (2006) Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol 7(2):131–142. https://doi.org/10.1038/nrm1835

Todo T, Usui M, Nagashima K (1992) Cerebral metastasis of malignant cardiac myxoma. Surg Neurol 37(5):374–379. https://doi.org/10.1016/0090-3019(92)90007-A

Tomifuji M, Imanishi Y, Araki K, Yamashita T, Yamamoto S, Kameyama K, Shiotani A (2011) Tumor depth as a predictor of lymph node metastasis of supraglottic and hypopharyngeal cancers. Ann Surg Oncol 18(2):490–496. https://doi.org/10.1245/s10434-010-1219-5

Vargas-Alarcón G, Vargas-Barrón J, Cruz-Robles D, Pérez-Vielma N, García-Trejo JJ, Aguilar-Gaytán R, Cortés-Hernández P, Vazquez-Ortíz ZY, Romero-Cardenas A (2008) A deletion in the PRKAR1A gene is associated with Carney complex. J Pediatr Endocrinol Metab 21(7):705–709. https://doi.org/10.1515/JPEM.2008.21.7.705

Vargas-Barron J, Romero-Cardenas A, Villegas M, Keirns C, Gomez-Jaume A, Delong R, Malo-Camacho R (1991) Transthoracic and transesophageal echocardiographic diagnosis of myxomas in the four cardiac cavities. Am Heart J 121(3 Pt 1):931–933. https://doi.org/10.1016/0002-8703(91)90216-5

Villalpando-Mendoza E, Pérez-Pérez FM, Cortés-Flores AO, Velázquez-Ramírez GA, Fuentes-Orozco C, González-Ojeda A (2006) Frequency of heart tumors and their histopathological correlation. Cir Cir 74(3):183–187

Wada A, Kanda T, Hayashi R, Imai S, Suzuki T, Murata K (1993) Cardiac myxoma metastasized to the brain: potential role of endogenous interleukin-6. Cardiology 83(3):208–211. https://doi.org/10.1159/000175971

Wang C, Lisanti MP, Liao DJ (2011) Reviewing once more the c-myc and Ras collaboration: converging at the cyclin D1-CDK4 complex and challenging basic concepts of cancer biology. Cell Cycle 10(1):57–67. https://doi.org/10.4161/cc.10.1.14449

Wang S, Cheng Y, Zheng Y, He Z, Chen W, Zhou W, Duan C, Zhang C (2016) PRKAR1A is a functional tumor suppressor inhibiting ERK/Snail/E-cadherin pathway in lung adenocarcinoma. Sci Rep 6:39630. https://doi.org/10.1038/srep39630

Wittersheim M, Heydt C, Hoffmann F, Büttner R (2017) KRAS mutation in papillary fibroelastoma: a true cardiac neoplasm? J Pathol Clin Res 3(2):100–104. https://doi.org/10.1002/cjp2.66

Wu XY, Liu WT, Wu ZF, Chen C, Liu JY, Wu GN, Yao XQ, Liu FK, Li G (2016) Identification of HRAS as cancer-promoting gene in gastric carcinoma cell aggressiveness. Am J Cancer Res 6(9):1935–1948

Yadav BS, Chanana P, Jhamb S (2015) Biomarkers in triple negative breast cancer: a review. World J Clin Oncol 6(6):252–263. https://doi.org/10.5306/wjco.v6.i6.252

Zhong H, De Marzo AM, Laughner E, Lim M, Hilton DA, Zagzag D, Buechler P, Isaacs WB, Semenza GL, Simons JW (1999) Overexpression of hypoxia-inducible factor 1alpha in common human cancers and their metastases. Cancer Res 59(22):5830–5835