A new clonal chromosomal aberration (47, XY, +21) in atrial myxoma from an elderly male patient

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Abstract

Myxomas are the most common primary cardiac tumors, with an estimated incidence of 0.5 per million per year. Familial myxoma constitutes 10% of all myxomas, among these tumors, one in ten is part of Carney complex - an autosomal dominant syndrome, which are related to some mutations in the PRKAR1A gene. We report a case of 75-year-old man with sporadic cardiac myxoma of a 4-cm large tumor, arising from the left side of the atrial septum and causing a severe left ventricle inflow obstruction. Cytogenetic analysis confirmed by fluorescence *in situ* hybridization method (FISH), demonstrated a numerical aberration in atrial myxoma cells: 47, XY, +21. Flow cytometry analysis demonstrated that a quarter of tumors cells were hematopoietic progenitor cells ($CD34^+$) and that a similar number were endothelial specific neovascular cells ($CD31^+$). These finding suggest that, hematopoietic progenitor cells may play an important role in the histogenesis of cardiac myxomas and the karyotype aberrations have an impact on sporadic tumor genesis. Nevertheless, genetic screening for sporadic (non-familial) cardiac myxomas is not recommended.

Introduction

Myxomas are the most common primary cardiac tumors, with an estimated incidence of 0.5 per million per year.[1] In Poland, between 2009 and 2011 the number of surgical procedures for cardiac neoplasms, increased from 119 to 152, where more than 90% of them were cardiac myxomas and 10% were other neoplasms such as rhabdomyo-, angio- and other sarcomas, or hematological tumour.[2] They are mainly diagnosed between the third and the sixth decade of life and they are more likely to occur in women than in men.[3-5] Asymptomatic course, with incidental diagnosis, is relatively frequent and it relates to about 11% of recognized tumors. The only effective treatment providing a chance for successful recovery and long-term survival is surgical excision.[6,7]

Familial myxomas constitute 10% of all myxomas, among these tumors one in ten are part of Carney complex - an autosomal dominant syndrome characterized by spotty pigmentation, cardiac and cutaneous myxomas, various endocrinal overactivity and neoplasms, which are related to some mutations in the PRKAR1A gene. PRKAR1A is a tumor suppressor gene localized on chromosome 17q22-24, that encodes for the protein kinase A regulatory 1-alpha subunit.[8,9] However, other chromosomal abnormalities, including those on chromosome 2 (2p16) are also possible.[10] Cytological examination of cells harvested from atrial myxomas revealed many non-clonal chromosomal abnormalities involving chromosomes: 1, 7, 9, 10, 12, 17 and 20.[11-13]

In this article we describe the chromosomal pattern of a case of atrial myxoma, showing a novel clonal numerical aberration. Additionally, we present the results of flow cytometry analysis of cells isolated from the tumor.

Case Report

In June 2009, a 75-year-old man of Polish descent was admitted to the emergency ward after an episode of fainting. In addition, the patient complained of shortness of breath accompanied by occasional palpitations and dizziness. His medical history revealed hypertension, coronary artery disease and Parkinson's disease. Physical examination revealed a high blood pressure (149/85 mm Hg) and a temperature of 37.2°C. Biochemical parameters on admission are presented in Table 1. The patient denied any history of cardiac tumors and there were no clinical features of familial myxomas (such as Carney complex or familial autosomal dominant syndrome). The presence of a 4-cm large tumor, arising from the left side of the atrial septum and causing a severe left ventricle inflow obstruction, was detected by 2-D echocardiography. Cardiac angiography performed before surgery showed critical stenosis in several coronaries, thus the patient qualified for urgent coronary by-pass grafting in combination with complete excision of the left atrial mass and a cuff of the inter-atrial septum. The operation was performed using the midsternotomy approach and standard operating procedures with repeated antegrade of cold crystalloid cardioplegia. Moderate hypothermia (30-32°C) was maintained during cardiac arrest. Postoperative biochemical parameters are presented in Table 1. During the perioperative period some episodes of seizures without symptoms of global central nervous system dysfunction were reported. The patient was treated with cefazolin during the perioperative period (4×1 g *i.v.*). Two days after surgery the patient developed a fever with increased inflammatory markers (CRP 207.1 mg/L) and 3×1.2 g i.v of amoxiclav was administered. The patient died on the 14th day after surgery due to multiple organ failure.

Pathological examination

Macroscopic examination revealed a gelatinous tumor, typically pedunculated with a stalk-shaped mass of tissue. The tumor was 4.2×3.8×2.9 cm in size, pale yellow, nodular with an irregular surface and focally hemorrhagic. Haematoxylin and eosin (HE) staining revealed elongated, round or stellate single cells and tenuous cords typically infiltrated by lymphocytes and macrophages and surrounded by myxoid stroma.

Cytogenetic study

After removing thrombi, the tumor tissue

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was gently dissected and digested for 48 hours at 37°C in collagenase Type II solution at a concentration of 200 U/mL (No. 17101-015, Invitrogen, Paisley, UK) dissolved in Dulbecco Modified Eagle Medium (GIBCO®DMEM, No. 11960-044, Invitrogen, Paisley, UK) supplemented with 10% Fetal Bovine Serum (GIBCO® FBS, No. 10100139, Invitrogen), 2 mM of L-glutamine (No. G8540, Sigma-Aldrich, Steinheim, Germany) and antibiotics (50 U/mL penicillin, 50 μ/mL streptomycin, 0.1 mg/mL neomycin, Polfa Tarchomin, Poland). Digested tissue, for cytogenetic analysis, was squeezed through a 70-μm nylon mesh into the DMEM supplemented with 10% FBS to create a single cell suspension, centrifuged (220 g, 10 min), washed and cultured in a 25ccm flask at 37°C in a humidified atmosphere (Figure 1). Mitotic arrest was achieved after 48 h of culturing by further overnight incubation with colcemid at a concentration of 0.01 μg/mL and GTG-banded metaphases were analysed. Complementary karyotype analysis was performed by fluorescence in situ hybridization method (FISH) with probes specific for LSI AML1(RUNX1). The analysis demonstrated a numerical aberration in atrial myxoma cells: 47, XY, +21 (Figure 2A, 2B). Because he had no phenotypical signs of Down syndrome a blood karyotype was not performed.

Flow cytometry analysis

The cell suspension was analyzed by means of the FACS Calibur instrument (BD Immunocytometry Systems, San Jose, USA). Cell-specific antibodies allowed the investigating of the hematopoietic phenotype of harvested cells: CD31 (PECAM-1) stained with fluorescein isothiocyanate (FITC, No. 560984, BD Bioscience; Erembodegem, Belgium) and CD34 stained with R-phycoerythrin (RPE, No. R712501, DakoCytomation; Glostrup, Denmark). The data was analysed using CellQuest Pro software (BD Immunocytometry Systems). It was observed that 26% of cells revealed hematopoietic progenitor potential – $CD34^+$ (Figure 2C) and 22% of them were positive in endothelial specific antigen – $CD31^+$ (Figure 2D).

Discussion

In this article, we report a case of an elderly male patient with a symptomatic atrial myxoma. Taking into consideration the age and the negative family history of the patient we assumed that our case was an example of a sporadic atrial neoplasm.

Histologically, cardiac myxomas are generally derived from subendocardial multipotential mesenchymal cells and imitate primitive mesenchyme.[14] In our study we confirmed that a quarter of tumors cells were hematopoietic progenitor cells ($CD34^+$) and that a similar number were endothelial specific neovascular cells ($CD31^+$). CD34 is a 105- to 120-kD transmembrane cell surface glycoprotein, which is selectively expressed at its highest level on the

Table 1. Pre- and postoperative biochemical parameters of a surgical patient for atrial myxoma and coronary artery stenosis.

	On admission before surgery	13th day after surgery
WBC [10^3cell/L]	10.5 H	9.8 H
Neutrophils [10^3cell/L]	7.7	9.0
Lymphocytes [10^3cell/L]	1.9	0.5
Monocytes, Basophils and others [10^3cell/L]	0.9	0.3
Erythrocytes [10^3cell/L]	3.88 L	2.42 L
Haemoglobin [g/dL]	11.9 L	7.8 L
Haematocrit [%]	35.5 L	22.9 L
MCV [fL]	91.5	94.6
MCH [pg]	30.7	31.4
PLT [10^3/L]	140.0	107 L
MPV [fL]	10.3	10.1
CRP [mg/L]	41.4 H	207.1 H
CK [U/l]	302 H	346 H *
CK-MB [U/l]	25 H	22 H *
cTnI [ng/mL]	5.91 H	3.56 H *
GFR	57,7 L	12.4 L
Glucose [mmol/L]	6.9 H	27 H
Urea [mmol/L]	7.7 H	23.7 H
Na^+ [mmol/L]	141	145
K^+ [mmol/L]	3.9	4.1

*Data collected during 2nd day after surgery. Abnormal values are marked as H – high or L – low according to a laboratory norm. CK, creatinine kinese; CK-MB, creatinine kinase muscle type izoenzyme; cTnI, cardiac troponin I; GFR, Glomerular filtration rate; kinase CRP, C-reactive protein; WBC, white blood cells; MCV, medium erythrocyte volume; MCH, medium haemoglobin weight in the erythrocyte; MPV, medium platelet volume; PLT, platelet number.

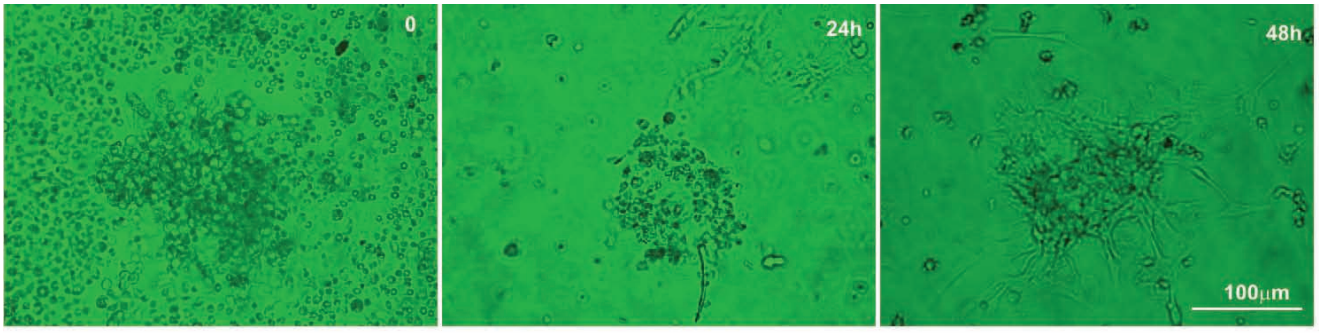

Figure 1. Primary cultures of cells harvested from an atrial myxoma excised from a symptomatic, 75-years-old-male patient. After collagenase Type II digestion, cells spontaneously formed aggregates and attached to a cell culture dish. Erythrocyte contamination was observed (0). On the first day of culturing, cells started to spread over the surface (24 h). After 48 h the cells presented their migratory phenotype.

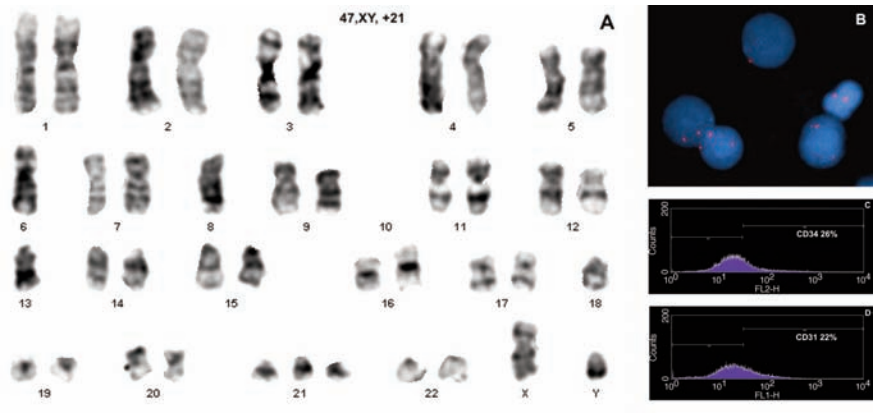

Figure 2. Cytogenetic and flow cytometry analysis of cells harvested from an atrial myxoma excised from a symptomatic, 75-years old male patient. The GTG-analysis revealed a clonal chromosomal aberration (47, XY, +21) (A), which was confirmed by fluorescence in situ hybridization method (B). Additionally, the phenotype of myxoma cells was analyzed and large populations of hematopoietic progenitor cells – CD34+ (C) and endothelial-like cells – CD31+ (D) were observed.

immature cells and decreases progressively with their development. CD34 antigen is a stage specific rather than a lineage-specific antigen, appears to be expressed on circulating peripheral blood cells in the adult and the number of $CD34^+$ cells is decreased in severe congestive heart failure.[15] These populations of hematopoietic progenitor cells ($CD34^+$) and endothelial specific neovascular cells ($CD31^+$) are likely derived from peripheral blood cells and may cover the tumor surface by monolayer to produce interspersed primitive blood vessels. These cells may also be dispersed in a myxoid matrix or form complex multilayer structures. However, the increased frequency of $CD34^+$ cells is characteristic for malignant neoplasms of hematopoietic stem cells.

Diagnostics of cardiac myxoma

Familial myxomas are rare; their frequency does not exceed 1 in 10 cases of cardiac tumors, both in European and Asian populations.[1,2,17] In the case of familial recurrent myxomas (Carney complex), their incidence appears to have autosomal dominant transmission with incomplete penetrance.[17,18] Despite the fact, that Carney complex can be characterized by specific clinical manifestations, treated family members require effective screening, including urinary free cortisol (Cushing syndrome), thyrotropin-releasing hormone, testicular or thyroid ultrasonography (other endocrinal abnormalities), routine echocardiographic screening and searches for cardiac and mucocutaneous myxomas in multiple locations. Thus, siblings (first-degree relatives) of an affected patient should be under medical care.[18] Additionally, genetic testing for the inactivation of the PRKAR1α gene (sequencing or higher resolution banding) is recommended.[17,18] In cases of more often, non-familial cardiac myxomas, we cannot endorse genetic screening.

Genetics of cardiac myxoma

Analysis of diseased specific chromosomal abnormalities is usually based on the demonstration of clonal chromosomal aberrations (CCAs). However, CCAs are rather indicative of the relative stability of the karyotype, whereas non-clonal chromosomal aberrations (NCCAs) are representative of an unstable genome.[19] Sometimes, non-recurrent chromosomal aberrations are more specific for cancer evolution. Despite extensive numbers of myxoma cell line analyses, it has been difficult to find recurrent chromosomal aberrations that are myxoma-specific.[11-13,20-23] Cytogenetic analysis revealed some clonal autosomal rearrangements: (17)(q10),der(20)t(1;20)(q21;q11.2), (9)(p22),+12,[12] add(1)(q32),[22] and others, however, only a translocation between chromosomes 1 and 12 (12p12) sporadically happened in another case.[13] Other defects include telomeric associated rearrangements focused on chromosomes 13 and 15 or loss of the Y chromosome. Cytogenetic analysis of myxomas in patients suffering from Carney complex has shown a role for regions of chromosome 2p16, however, cytogenetic analysis cannot confirm a role for 2p16 in cases of sporadic myxoma.[10] Nevertheless, a limited involvement in structural rearrangement for chromosome 17q2 has been proved in sporadic cardiac myxomas.[11,12]

In our study we observed an acquired clonal trisomy 21 in cardiac myxoma cells found as a single karyotype aberration. Trisomy 21 as an acquired clonal aberration has been previously reported in human neoplasms.[24] Moreover, germinal trisomy 21 predisposes to two related hematopoietic diseases: transient myeloproliferative disorder (TMD) and acute megakaryoblastic leukemia (AMKL).[25] However, the assessment of the possible discordance of phenotype between normal tissues and cancer cells created by karyotypic abnormalities was not performed in our patient.

Conclusion

Hematopoietic progenitor cells may play an important role in the histogenesis of cardiac myxomas and the karyotype aberrations have an impact on sporadic tumor genesis of the heart. There are no stage or cancer type specific recurrent chromosomal aberrations in sporadic cardiac myxoma. Genetic screening for sporadic (non-familial) cardiac myxomas is not recommended.

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