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Brief Definitive Report

KRAS mutation in papillary fibroelastoma: a true cardiac neoplasm?

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Abstract

Primary cardiac tumours are rare and mostly benign lesions. Recent publications report that cardiac papillary fibroelastomas are the most common benign primary heart tumour, outnumbering myxomas. However, there is no consensus about their aetiology. We investigated the molecular profile of these tumours using next generation sequencing in a cohort of 16 cases. Eleven of 14 (79%) analysable tumours showed mutations of the *KRAS* oncogene. Our results provide unambiguous evidence that a significant proportion of these lesions are genuine neoplastic tumours caused by an oncogenic driver mutation.

Keywords: primary cardiac tumours; papillary fibroelastoma; *KRAS* mutation

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Introduction

Primary cardiac tumours are exceptionally rare; in a meta-analysis of several large autopsy series a frequency of approximately 0.02% was detected [1]. About 75% of these tumours can be classified as benign and 25% as malignant [1]. Of the benign tumours, myxomas have been considered to be the most common entity, followed by lipomas and papillary fibroelastomas [1–3]. However, contrary to the common doctrine, a recent publication with a large collection of the latter concluded that they might actually be more prevalent than myxomas [4]. Yet there is no consensus about the aetiology of papillary fibroelastomas, and some authors classify this entity as a 'tumour-like lesion' rather than a genuine neoplasm [5–9].

In this paper we present evidence that a large proportion of these lesions show oncogenic alterations in the *KRAS* gene and thus should be considered as true neoplasms.

Methods

Between the years of 2010 and 2016 a total of 16 cardiac papillary fibroelastomas were diagnosed in the Institute of Pathology of the University Hospital Cologne. With approval of the ethics committee the histological slides and paraffin embedded tissue samples were retrieved from the archive and re-evaluated.

Six sections of 10 μm thickness were cut from each of the formalin-fixed and paraffin-embedded tissue samples and subsequently deparaffinised. The tumour areas were macro-dissected from unstained slides using a marked haematoxylin-eosin (H&E) stained slide as a reference. After proteinase K digestion, DNA was isolated with the Maxwell® 16 FFPE Plus Tissue LEV DNA Purification Kit (Promega, Mannheim, Germany) on the Maxwell® 16 device (Promega) following the manufacturer's instructions. The DNA content was quantified and assessed for quality using quantitative real-time PCR (qPCR).

Multiplex PCR-based target enrichment was performed as described in detail previously [10]. Isolated DNA was amplified with an Ion AmpliSeq Custom DNA Panel (Thermo Fisher Scientific, Waltham, MA, USA) targeting 14 lung cancer genes, and the Ion AmpliSeq Library Kit 2.0 (Thermo Fisher Scientific) according to the Ion AmpliSeq Library Preparation User Guide (Thermo Fisher Scientific). The panel comprises a subset of cancer relevant genes including: *AKT1*, *ALK*, *BRAF*, *CTNNB1*, *DDR2*, *EGFR*, *ERBB2*,

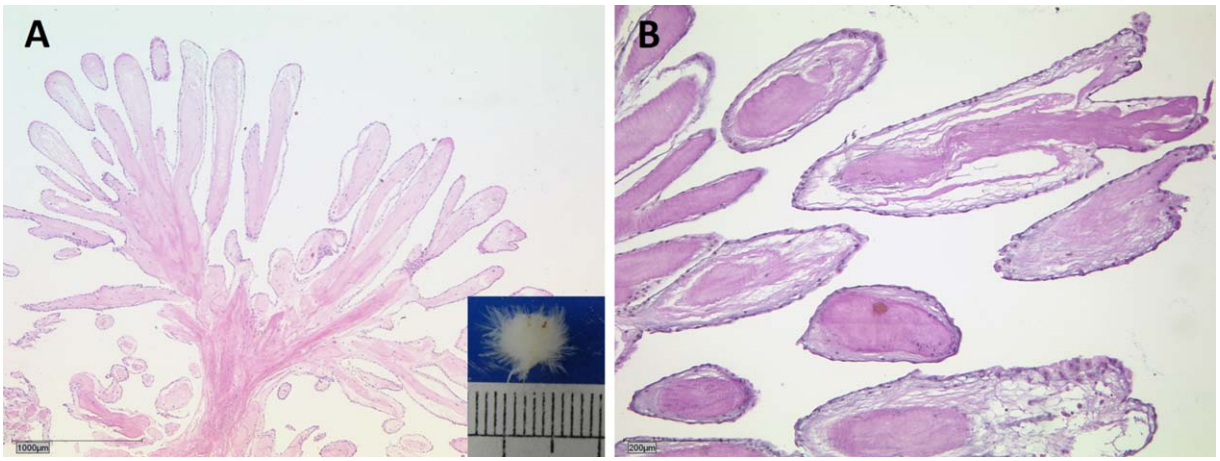

Figure 1. Macroscopic and microscopic characteristics of papillary fibroelastomas: (A) Microscopically avascular tumours with numerous papillary fronds attached to the endocardium by a short stalk. H&E staining, 25× magnification, macroscopically distinctive 'sea anemone-like' appearance (insert bottom right). (B) The papillary fronds are lined by endothelium and show a central core of dense, hyalinised connective tissue surrounded by loose connective tissue. EvG staining, 200× magnification.

KRAS, *MAP2K1*, *MET*, *NRAS*, *PIK3CA*, *PTEN* and *TP53*.

Libraries were constructed using the Gene Read DNA Library I Core Kit and the Gene Read DNA I Amp Kit (Qiagen, Hilden, Germany). After end-repair and adenylation, NEXTflex DNA Barcodes were ligated (Bio Scientific, Austin, TX, USA). Barcoded libraries were amplified and then the final library product was quantified with Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific) on the Qubit 2.0 Fluorometer (Thermo Fisher Scientific), diluted and pooled in equal amounts. Finally, 12 pM of the constructed libraries were sequenced on the MiSeq (Illumina, San Diego, CA, USA) with a MiSeq reagent kit V2 (300-cycles) (Illumina) following the manufacturer's recommendations.

Data were exported as FASTQ files. Alignment and annotation was done using a modified version of a previously described method [11]. BAM files were visualised in the Integrative Genomics Viewer (http://www.broadinstitute.org/igv/, Cambridge; USA). A 5% cut-off for variant calls was used and results were only interpreted if the coverage was >200×.

Results

Our cohort consisted of 16 patients with papillary fibroelastoma. The mean age of the patients was 64 years (range 42–80 years); nine (56%) were male and seven (44%) were female. Eleven (69%) of the tumours affected the valves, the aortic valve being the most common localisation. Fourteen samples were analysable. Of these 11 (79%) showed point mutations in *KRAS*. Seven (64%) of them were male, four female (36%). The majority of the mutations (75%) were located in codon 12 of *KRAS* exon 2, the remainder at codon 61 of *KRAS* exon 3. Three patients showed a wildtype sequence (21%). Two of the 16 samples could not be analysed due to sequencing artefacts which occurred after PCR amplification and can be attributed to formalin fixation. Although these two tumours were of medium size and had a high enough tumour cell content, the samples had a very low DNA concentration as well as very poor DNA quality.

No other genomic alterations were detected with the panel described above. Figure 1 shows the macroscopic and histomorphological characteristics of papillary fibroelastomas, Figure 2 shows the typical echocardiographic appearances. Patients' characteristics and their mutational status are listed in Table 1.

Discussion

Papillary fibroelastomas are the most common primary tumours of the cardiac valves [9,12,13]. A recent publication from Tarmin *et al*., analysing frequency and clinical course of papillary fibroelastoma in a cohort of 511 patients came to the conclusion that they are more common than myxomas, thus being the most frequent benign primary heart tumour overall [4]. The majority of papillary fibroelastomas (80–90%) occur on the heart valves, with the aortic valve being the single most common localisation, but

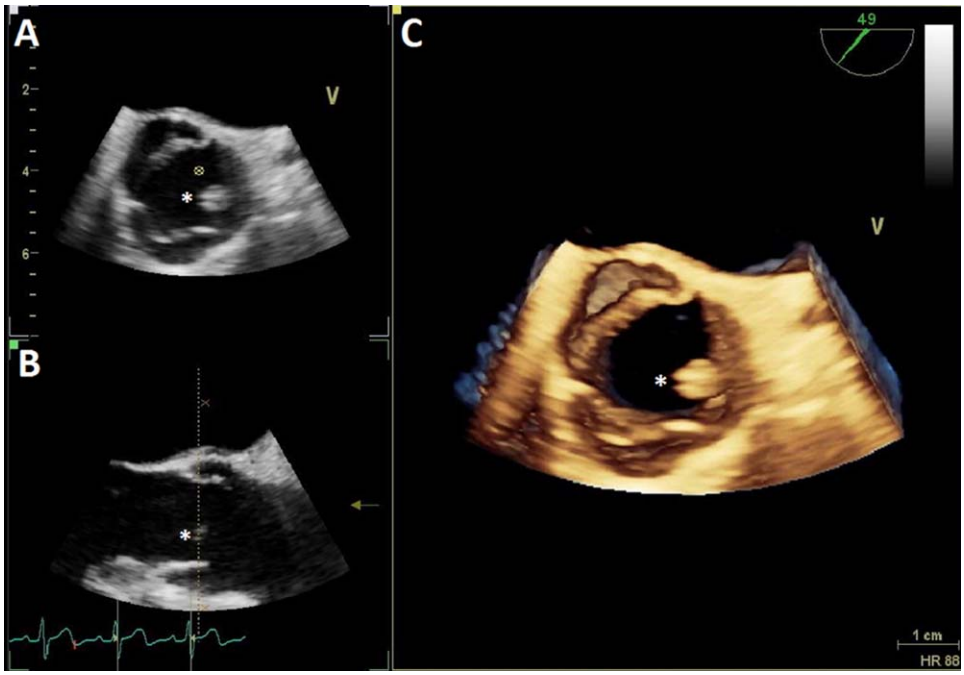

Figure 2. Transesophageal echocardiography of a papillary fibroelastoma (asterisks) of the left coronary leaflet of the aortic valve: (A) Atypical short axis view of 49°. (B) Long axis view showing the left ventricular outflow tract, the aortic valve, the fibroelastoma and the aortic bulb. (C) Three-dimensional reconstruction of the aortic valve.

they can arise elsewhere in the atria and ventricles [4,13–17]. The mean age at detection is approximately 60 years, and a slight male predominance has been described [13,16].

Resembling sea anemones at low magnification, papillary fibroelastomas are pedunculated, avascular structures with numerous papillary fronds [12]. Histologically they are lined by endothelium and show a central core of dense, hyalinised connective tissue surrounded by an intermediate layer of proteoglycan-rich loose connective tissue mingled with elastic fibres [9,12] (Figure 1). Their size can range from 2 to 70 mm in greatest diameter [13].

In most cases papillary fibroelastomas are discovered incidentally during medical examinations; in the past they were incidental findings at autopsy or heart surgery for other indications [18,19]. With the frequent application of echocardiography and transoesophageal echocardiography, and the improvement in quality in these imaging techniques over the last

Table 1. Patients' characteristics and *KRAS* mutation status

Age	Sex	Localisation	*KRAS* Mutation	Allele Frequency (%)
62	F	Mitral valve	c.183A>T p.Q61H	13.6
62	F	Mitral valve	c.183A>C p.Q61H	17.8
63	M	Mitral valve	c.34G>T p.G12C	12.3
74	M	Left atrium	c.34G>T p.G12C	47.9
68	M	Mitral valve	c.35G>A p.G12D	55.9
72	F	LVOT	c.35G>A p.G12D	66.6
57	M	Atrium	c.35G>A p.G12D	34.9
51	M	Aortic valve	c.35G>T p.G12V	20.3
42	F	Aortic valve	c.35G>T p.G12V	12
76	M	Tricuspid valve	c.35G>T p.G12V	50.5
76	F	LVOT	c.35G>C p.G12A	32.2
70	M	Aortic valve	Wildtype	-
53	F	Aortic valve	Wildtype	-
80	F	Aortic valve	Wildtype	-
62	M	Left auricle	na	na
59	M	Aortic valve	na	na

LVOT = left ventricular outflow tract; na = not analysable.

decades, there has been a shift from postmortem to antemortem diagnosis, and the diagnosis of papillary fibroelastoma is made more often [4,13,15,16].

Clinically most papillary fibroelastomas are asymptomatic, but they possess embolic potential leading to life-threatening complications such as coronary and cerebral embolism with consequent ischaemic damage or stroke and thus should be resected [2,4,13,17].

There is controversy about the aetiology of papillary fibroelastomas. The observation that the papillary fronds are similar in structure to normal chordae tendineae was interpreted as evidence that they might be of hamartomatous origin [18]. Salyer and colleagues proposed development from organised mural thrombi as a result of endocardial damage [5]. Single cases have been described in the context of rheumatic disease [14,20]. An association with hypertrophic (obstructive) cardiomyopathy has been reported by different authors [6,14–16,19] and proposed as a predisposing factor [16]. Other associated endocardial abnormalities described previously include degenerative aortic valve thickening or sclerosis, bicuspid valve stenosis and mitral valve prolapse [14,15,17]. In an immunohistological study of four papillary fibroelastomas, Grandmougin *et al*. found dendritic cells and cytomegalovirus (CMV) remnants, suggesting virus-induced tumour growth in the context of a chronic form of viral endocarditis [21].

Multiple single case reports and studies in small cohorts have described the occurrence of papillary fibroelastomas in patients who had undergone previous open-heart surgery for different, unrelated indications [3,6,14,16,19,20,22]. Furthermore an association with radiotherapy [6,14] has been reported in a couple of cases. These findings have led to the assumption that at least a proportion of papillary fibroelastomas might be acquired, reactive lesions.

In this context it has been hypothesised that, aside from the procedure itself, continuing turbulent blood flow in the heart and consequent haemodynamic trauma of the endothelium contributes to the development of papillary fibroelastomas [3,6]. Yet endocardial damage is frequent whereas papillary fibroelastomas are uncommon and, since the latency period between surgery and development of papillary fibroelastomas in these so-called 'iatrogenic' cases ranges from 8 to 31 years [6,19,22], it cannot be ruled out that the correlation might be coincidental rather than causal.

In light of this controversy, the 4th edition of the WHO Classification of tumours of the lung, pleura, thymus and heart states that there is no histological or molecular proof to support a true neoplastic origin of papillary fibroelastomas [9]. Our analysis of a cohort of 16 papillary fibroelastomas revealed very frequent, recurrent and unambiguously oncogenic *KRAS* mutations. Thus, papillary fibroelastomas represent true neoplastic lesions of limited growth potential. *KRAS* mutations occur in various tumours and are amongst the most frequent oncogenic driver lesions present both in malignant and also in benign tumours. In small cohorts and single cases *KRAS* mutations have been described in primary cardiac sarcomas [23,24] but to the best of our knowledge this is the first report of *KRAS* mutations in cardiac papillary fibroelastomas.

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Author contributions

MW conceived the study, acquired the collective, interpreted the data and wrote the manuscript. CH performed the sequencing, analysed the data and helped preparing the manuscript. FH generated a figure and critically revised the manuscript. RB conceived the study, interpreted the data and critically revised the manuscript.

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