KRAS mutation in papillary fibroelastoma: a true cardiac neoplasm?

Maike Wittersheim,¹* Carina Heydt,¹ Fabian Hoffmann² and Reinhard Böttner¹

¹Institute of Pathology, University Hospital of Cologne, Cologne, Germany
²Department of Internal Medicine III, Heart Center, University Hospital of Cologne, Cologne, Germany

*Correspondence to: Maike Wittersheim, Institute of Pathology, University Hospital of Cologne, Kerpener Str. 62, 50931 Cologne, Germany. E-mail: maike.wittersheim@uk-koeln.de

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

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,
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 fibroelastomas. 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
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.

Acknowledgements

The authors would like to thank Jesko Cordes for his help in preparing the figures and Stephan C. Schäfer for his careful and critical reading of the manuscript.

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. All authors had final approval of the submitted version.

References

1. Reynen K. Frequency of primary tumors of the heart. Am J Cardiol 1996; 77: 107.
2. Khair T, Mazidi P, Laos LF. Cardiac papillary fibroelastoma: case report and review of the literature. Int J Cardiol 2010; 139: 102–104.
3. Okamoto Y, Matsumoto M, Inoue H, et al. Aortic valve papillary fibroelastoma that developed rapidly after open-heart surgery. Interact Cardiovasc Thorac Surg 2008; 7: 1134–1136.
4. Tamin SS, Maleszewski JJ, Scott CG, et al. Prognostic and bioepidemiologic implications of papillary fibroelastomas. J Am Coll Cardiol 2015; 65: 2420–2429.
5. Salyer WR, Salyer DC. Myxoma-like features of organizing thrombi in arteries and veins. Arch Pathol 1975; 99: 307–311.
6. Kurup AN, Tazelaar HD, Edwards WD, et al. Iatrogenic cardiac papillary fibroelastoma: a study of 12 cases (1990 to 2000). Hum Pathol 2002; 33: 1165–1169.
7. Miller DV, Edwards WD. Cardiovascular tumor-like conditions. Semin Diagn Pathol 2008; 25: 54–64.
8. Fleischmann KE, Schiller NB. Papillary fibroelastoma: move over myxoma. J Am Coll Cardiol 2015; 65: 2430–2432.

© 2017 The Authors The Journal of Pathology: Clinical Research published by The Pathological Society of Great Britain and Ireland and John Wiley & Sons Ltd
9. Travis WD, Brambilla E, Allen P, Burke, Alexander Marx, Andrew G. Nicholson (Eds). World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart 4th Edn. IARC Press, Lyon, 2015.
10. König K, Peifer M, Fassunke J, et al. Implementation of amplicon parallel sequencing leads to improvement of diagnosis and therapy of lung cancer patients. J Thorac Oncol 2015; 10: 1049–1057.
11. Peifer M, Fernandez-Cuesta L, Sos ML, et al. Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer. Nat Genet 2012; 44: 1104–1110.
12. Edwards FH, Hale D, Cohen A, et al. Primary cardiac valve tumors. Ann Thorac Surg 1991; 52: 1127–1131.
13. Gowda RM, Khan IA, Nair CK, et al. Cardiac papillary fibroelastoma: a comprehensive analysis of 725 cases. Am Heart J 2003; 146: 404–410.
14. Klarich KW, Enríquez-Sarano M, Gura GM, et al. Papillary fibroelastoma: echocardiographic characteristics for diagnosis and pathologic correlation. J Am Coll Cardiol 1997; 30: 784–790.
15. Sun JP, Asher CR, Yang XS, et al. Clinical and echocardiographic characteristics of papillary fibroelastomas: a retrospective and prospective study in 162 patients. Circulation 2001; 103: 2687–2693.
16. Ngaage DL, Mullany CJ, Daly RC, et al. Surgical treatment of cardiac papillary fibroelastoma: a single center experience with eighty-eight patients. Ann Thorac Surg 2005; 80: 1712–1718.
17. Val-Bernal JF, Mayorga M, Garrio MF, et al. Cardiac papillary fibroelastoma: retrospective clinicopathologic study of 17 tumors with resection at a single institution and literature review. Pathol Res Pract 2013; 209: 208–214.
18. McAllister HA, Fenoglio JJ. Tumors of the Cardiovascular System. In Atlas of Tumor Pathology, Series 2. Armed Forces Institute of Pathology, Washington DC, 1978.
19. Lee KS, Topol EJ, Stewart WJ. Atypical presentation of papillary fibroelastoma mimicking multiple vegetations in suspected subacute bacterial endocarditis. Am Heart J 1993; 125: 1443–1445.
20. Cha SD, Incarvito J, Fernandez J, et al. Giant Lambli’s excrescences of papillary muscle and aortic valve: echocardiographic, angiographic, and pathologic findings. Clin Cardiol 1981; 4: 51–54.
21. Grandmougin D, Fayad G, Moukassa D, et al. Cardiac valve papillary fibroelastomas: clinical, histological and immunohistochemical studies and a physiopathogenic hypothesis. J Heart Valve Dis 2000; 9: 832–841.
22. Levinsky L, Srinivasan V, Gingell RL, et al. Papillary fibroelastoma of aortic and mitral valves following myectomy for idiopathic hypertrophic subaortic stenosis. Thorac Cardiovasc Surg 1981; 29: 187–191.
23. Garcia JM, Gonzalez R, Silva JM, et al. Mutational status of K-ras and TP53 genes in primary sarcomas of the heart. Br J Cancer 2000; 82: 1183–1185.
24. Wittersheim M, Böll B, Shimabukuro-Vornhagen A, et al. Extensively metastasized cardiac angiosarcoma mimicking systemic sarcoïdosis. Virchows Arch 2016; 469; S242.