Detection of microsatellite instability in sporadic cardiac myxomas

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Abstract

Objective: Microsatellite instability (MIN) is an early event in DNA repair-deficient associated diseases and reflects an elevated mutation rate in the genome of neoplastic cells. Sporadic cardiac myxomas are the most common primary heart tumours and their aetiopathology remains obscure. This study investigates the incidence of MIN in sporadic cardiac myxomas as a possible genetic mechanism of tumour pathogenesis.

Methods: Eleven surgically excised sporadic cardiac myxomas were assessed for MIN using twenty-two highly polymorphic microsatellite markers, located on a wide range of chromosomal arms. DNA was extracted from myxoma tissue specimens as well as the respective normal tissue and subjected to polymerase chain reaction.

Results: The microsatellite analysis revealed that seven myxoma specimens (64\%) exhibited MIN in at least one marker. One tumour specimen exhibited evidence of MIN in four microsatellite markers, while the most frequently affected marker was D17S855 (27\%), located on chromosome 17q.

Discussion: We have detected a considerable incidence of MIN in sporadic cardiac myxomas indicating that decreased fidelity in DNA replication and repair is common in these tumours. To the best of our knowledge this is the first report describing MIN in sporadic cardiac myxomas, as a possible pathogenetic mechanism of these rare neoplasms. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Cardiac myxomas; Heart tumours; Microsatellite instability; Molecular biology; Polymerase chain reaction; Experimental; Heart

1. Introduction

The incidence of primary tumours of the heart in autopsy series ranges from 0.0017 to 0.28 per cent [1]. Cardiac myxomas are the most common primary heart neoplasms comprising 40\% of the tumors in most pathological series [2]. They may occur at any age, although 50\% occur in patients between 30 and 60 years of age [3]. These neoplasms may appear most often with one of three major features: obstruction of intracardiac blood flow, constitutional clinical manifestations or systemic arterial embolism. Histologically, cardiac myxomas consist of a myxoid matrix that contains relatively few polygonal cells with eosinophilic cytoplasm and many blood vessels. At the subcellular level, the only molecular data concerning familial cardiac myxomas have been found in Carney syndrome [4]. The origin of cardiac myxomas has been disputed. Ultrastructural findings suggest a multipotential mesenchymal cell as the origin. A strong positivity for factor VIII-related antigen favors the endothelial/endocardial origin, but co-expression of many other markers supports the first hypothesis [5]. Two theories exist: one claims that myxomas are thrombogenic in origin; the other considers them to be neoplastic. The presence of different nonclonal chromosomal rearrangements have been shown, supporting the theory for a neoplastic origin. However the etiopathology of these rare neoplasms remains obscure.

A recently discovered feature of the neoplastic cells is the elevated mutational rate which is reflected in the instability of the microsatellite DNA (microsatellite in-
stability, MIN) [6], MIN was initially reported in colorectal cancer (HNPCC) [7–9] and later extended to almost all human tumours [10–13], neurodegenerative diseases [14,15], atherosclerotic tissues [16], human pterygia [17] as well as spontaneously aborted embryonic tissues [18–20].

The aim of our study was to analyse the incidence of MIN in sporadic cardiac myxomas. If the molecular basis of the disease is similar to the development of neoplasia, then MIN should be expected to be present in this lesion and might also be associated with the presence of transforming oncogenes. Our data show that MIN occurs in cardiac myxomas at a considerable percentage and that an elevated mutational rate may be associated with the development of the disease.

2. Materials and methods

2.1. Specimens and DNA extraction

Eleven sporadic cardiac myxoma specimens were obtained from the ‘Evagelismos’ Hospital, Athens. Informed consent was obtained from all the patients who participated in this study. The clinical features of the patients examined are presented in Table 1, including sex, age location of the tumour and tumour size. Macroscopically, the lesions were pedunculated and papillary in seven patients and smooth and round in four patients. Light microscopic examination showed the typical features of cardiac myxoma in all 11 patients [21]. There was a myxomatous stroma with vascular channels and scattered immune cells such as macrophages, neutrophils and lymphocytes. Tumour cells were embedded in the stroma and were mostly polygonal but some were spindle-shaped. All tumours showed a benign behaviour with no metastasis. Tissue specimens were obtained from paraffin blocks. A matched normal DNA control from the adjacent normal cardiac tissue was obtained from the `Evagelismos' Hospital, Athens. Informed consent was obtained from all the patients who participated in the study.

Genomic DNA was extracted from the fixed tissues as previously described [10] and stored at 4°C until PCR amplification.

2.2. PCR amplification, microsatellite analysis

Twenty-two microsatellite markers (Research Genetics, USA) located on a wide range of chromosomal arms were used (Table 2). The vast majority of the markers used were comprised of dinucleotides. The only exceptions were markers TPO and HRM which were comprised of tetra- and hexanucleotides, respectively. The marker ANGIOTEN is located on chromosome 1, D2S103, D2S105 and TPO on chromosome 2, D3S1210, D3S1234 and D3S647 on chromosome 3, D6S344 on chromosome 6, D7S479 and D7S520 on chromosome 7, D8S133, PLAT2 and ANK1 on chromosome 8, D9S51 and D9S112 on chromosome 9, HRM on chromosome 11, D17S250, THRA1, D17S855 and D17S579 on chromosome 17, D18S43 on chromosome 18 and D19S49 on chromosome 19 (http://gdbwww.gdb.org). PCR analysis was performed in a 50-μl reaction volume containing 200 ng of genomic DNA, 1 μM of each primer, 250 μM dNTPs, 5 μl of 10X buffer (670 mM Tris-HCl, pH 8.5; 166 mM ammonium sulphate; 67 mM magnesium chloride; 1.7 mg/ml BSA; 100 μM β-mercaptoethanol and 1% (w/v) Triton X-100) and 1 U of Taq DNA polymerase. The reactions were denatured for 5 min at 95°C and the DNA was subsequently amplified for 30 cycles at 95°C for 30 s, 57°C for 40 s and 72°C for 30 s each step. Seven μl of the PCR product was electrophoresed in a 10% polyacrylamide gel and silver stained. MIN was scored by comparing the electrophoretic pattern of the microsatellite markers amplified from the paired DNA preparations (pathological/normal tissue), demonstrating a shift of one or both of the alleles in the pathological DNA specimen. The shift resulted from either an addition or deletion of one or more repeat units resulting in the generation of novel microsatellite alleles. In some cases, an appearance of a band, distinct from the other alleles was detected. The appearance of novel bands may be explained as the result of alterations in the length of the microsatellite repeats.

Table 1
Clinical features of patients with sporadic cardiac myxomas

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Tumour location</th>
<th>Tumour size (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>26</td>
<td>Left ventricle</td>
<td>8.7x3.3</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>57</td>
<td>Left atrium</td>
<td>7.5x2.7</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>56</td>
<td>Left atrium</td>
<td>10.4x4.0</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>67</td>
<td>Left atrium</td>
<td>7.4x1.5</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>48</td>
<td>Left atrium</td>
<td>6.5x4.2x3.5</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>47</td>
<td>Left atrium</td>
<td>9.3x3.5</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>46</td>
<td>Left atrium</td>
<td>7.6x1.3</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>55</td>
<td>Left atrium</td>
<td>6.1x3.8x3.1</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>44</td>
<td>Left atrium</td>
<td>5.2x3.5x1.5</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>52</td>
<td>Left atrium</td>
<td>4.5x3.5x2.5</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>48</td>
<td>Left atrium</td>
<td>6.5x4.0</td>
</tr>
</tbody>
</table>

Table 2
Mutations in microsatellite markers in sporadic cardiac myxomas

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>MIN affected markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D7S520</td>
</tr>
<tr>
<td>3</td>
<td>D17S855, D17S579, ANK1, D19S49</td>
</tr>
<tr>
<td>5</td>
<td>D7S479, D3S647</td>
</tr>
<tr>
<td>6</td>
<td>D17S855</td>
</tr>
<tr>
<td>8</td>
<td>D2S105, D17S250</td>
</tr>
<tr>
<td>9</td>
<td>D17S855</td>
</tr>
<tr>
<td>11</td>
<td>D7S520</td>
</tr>
</tbody>
</table>

The microsatellite markers analyzed were ANGIOTEN, D2S103, D2S105, TPO, D3S1210, D3S1234, D3S647, D6S344, D7S479, D7S520, D8S133, PLAT2, ANK1, D9S51, D9S112, HRM, D17S250, THRA1, D17S855, D17S579, D18S43, D19S49.
of microsatellites, limited only to a cellular subpopulation of the pathological tissue, creating novel cellular clones.

Thus, in a microsatellite analysis in total extracted DNA from the neoplastic tissue, one may observe alleles from both affected and unaffected microsatellites. The analysis in the MIN positive cases was repeated at least twice and the results were highly reproducible.

3. Results

Eleven surgically excised cardiac myxomas were analysed for the incidence of MIN using twenty-two microsatellite markers located on different chromosomal regions, representing loci that show a variable degree of alterations. Seven out of eleven specimens (64%) exhibited MIN in at least one microsatellite marker. All positive specimens for MIN and their corresponding microsatellite markers affected are presented in Table 2. Representative examples of specimens with MIN are shown in Fig. 1. The majority of the positive specimens (four among seven) were affected in one marker, two specimens exhibited evidence of MIN in two markers while one specimen was affected in four markers, reflecting a greater degree of genome destabilization. No association was found between the presence of MIN and the patients age or the tumour size. Interestingly, three out of the four male patients exhibited MIN.

4. Discussion

The knowledge concerning the genetic constitution of cardiac myxomas is quite limited. The heart myxomas are of particular interest as they are the most frequent primary neoplasm of the heart. However, genetic analyses in the microsatellite DNA of sporadic myxomas have not been performed to any large extent, therefore we investigated the relation between microsatellite DNA and the etiopathology of the disease. To our knowledge, there is only one report indicating the presence of frequent nonrandom telomeric translocations bearing a striking resemblance to that of other solid tumors [20]. In our study an additional genetic alteration affecting the length of microsatellite DNA in 64% of the cases of sporadic myxomas was found.

Genetic alterations such as microsatellite instability have been detected in almost all human tumours as well as in other diseases such as human atherosclerotic plaques [16] and human pterygia [17], leading to the suggestion that these diseases possess similarities with neoplasia.

MIN is an early event in the DNA repair-deficient diseases and corresponds to an elevated mutational rate of the cell. The precise significance of MIN observed still remains obscure as the information on the genetic basis of the disease is limited. However, we may postulate that the relatively high mutational rate of the sporadic heart myxomas, as reflected in the instability of the microsatel-

Fig. 1. Representative examples of specimens exhibiting MIN. N, normal DNA; T, tumour DNA. In all cases a shift in the mobility of the microsatellites or a generation of a novel allele is obvious and thus the specimens were scored as positive for MIN. The numbers above the locus name represent the patient numbers.
Skin myxomas known. These familial cardiac tumours [16] Spandidos DA, Ergazaki M, Arvanitis D, et al. Microsatellite

Tri or tetranucleotide microsatellites. In our study, mutations in simple repeated sequences reveal a new mechanism for
50-fold greater than that for dinucleotide microsatellites [1996. p. 2183±2184.

MIN, specimens unstable for
$2$ loci as `low' MIN. It previously has been shown that the rate of spontaneous changes in short tandem repeats of six bases is threefold higher than that in tandem repeat units comprised of two bases [28]. In addition, the rate of spontaneous mutation in individual tri- and tetranucleotide microsatellite markers can be as high as 50-fold greater than that for dinucleotide microsatellites [29,30]. Therefore, the two systems (di-versus tri- and tetranucleotide microsatellites) are not directly comparable and dinucleotide microsatellites are likely to be more useful as monitors of underlying genomic instability than tri or tetranucleotide microsatellites. In our study, using dinucleotide microsatellites we possibly underestimate the colonic carcinogenesis. Nature 1993;363:558±561.

We suggest that MIN is associated in the development of sporadic heart myxomas. Future studies involving the evaluation of the clinical significance of this phenomenon as well as the molecular mechanism and consequences of MIN may provide clues for the pathogenesis of the disease.

References


