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A primary cardiac leiomyosarcoma with mutation at H-*ras* codon 12

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Abstract. The presence of activating *ras* mutations in a cardiac leiomyosarcoma which occurred in the right atrium of the heart of a female patient was examined. The tumor had the appearance of leiomyosarcoma in rutine histopathological examination and the definite diagnosis was confirmed by a positive immunohistochemical reaction to smooth muscle actin. Molecular analysis by polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) technique showed a point mutation of H-*ras* gene at codon 12. To the best of our knowledge, this is the first report describing *ras* gene mutation in a cardiac leiomyosarcoma implying a role for the *ras* oncogenes in the development of this tumor.

Introduction

Primary cardiac neoplasms, both benign and malignant, are unusual tumors and are found in only about 0.002% of autopsies (1). About 25% of primary cardiac neoplasms are malignant and these are mainly sarcomas and lymphomas. The sarcomas most often occur at the base of the heart, involving preferentially the right atrium (2). In this report, we describe a case of a right atrial leiomyosarcoma afflicting a female patient, which showed in molecular analysis an H-*ras* gene mutation at codon 12.

Case report

The patient, a 46-year old woman, was admitted to the hospital complaining of increasing weakness and shortness of breath. The physical examination showed signs of congestive heart failure and the subsequent echocardiographic study and CT scan revealed a tumor arising in the right atrium of the heart. The patient underwent surgery and part of the tumor was removed. The pathological examination of the tumor showed a leiomyosarcoma composed of spindle cells arranged in interlacing bundles (3). The neoplastic cells had hyperchromatic nuclei and showed increased mitotic activity. Immunohistochemistry revealed that the tumor cells contained abundant smooth muscle cell actin (Fig. 1).

After the extraction of DNA from leiomyosarcoma tissue samples, we examined the presence of point mutations in codon 12 of H-*ras*, in codon 12 of K-*ras* and in codon 61 of N-*ras* by PCR-RFLP analysis using gene specific primer sets (4). The PCR products were digested by restriction endonucleases: MspI for H-*ras* codon 12 (overnight at 37°C), Bst NI for K-*ras* codon 12 (3 h at 60°C) and MscI for N-*ras* codon 61 (overnight at 37°C). Digestion products were electrophoresed through a 6% agarose gel for K-*ras* and H-*ras* or an 8% native polyacrylamide gel for N-*ras* and compared to appropriate controls. Gels were stained with ethidium bromide and visualized on a UV transilluminator.

The PCR amplification gave the expected sizes of 312 bp for codon 12 of H-*ras*, 157 bp for codon 12 of K-*ras* and 65 bp for codon 61 of N-*ras*. After the application of RFLP analysis, digestion products with sizes 291 bp (mutant) and 236 bp (normal) for codon 12 of H-*ras* (Fig. 2, lane 3) for the leiomyosarcoma case and 236 (normal) for codon 12 of H-*ras* (Fig. 2, lanes 2 and 4) for normal tissue were obtained. The respective products for codons 12 of K-*ras* and N-*ras* genes were 113 bp and 44 bp indicating in both cases the presence of normal alleles (data not shown).

Discussion

Carcinogenesis is a multistage process involving the activation of proto-oncogenes and the inactivation of tumor supressor genes (5). The H-*ras,* K-*ras* and N-*ras*, members of the *ras* family code for structurally and immunologically related proteins which are located on the inner side of the plasma membrane, possess GTPase activity and are thought to be part of a signal transduction pathway (6). Activating mutations of the *ras* family genes which occur mainly at codons 12 and 61, have been detected in a variety of human tumors. Generally, *ras* mutations are considered as early events in carcinogenesis and have been described in malignant tumors, leading to the proposal that they may serve as tumor markers (7).

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Abbreviations: PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism

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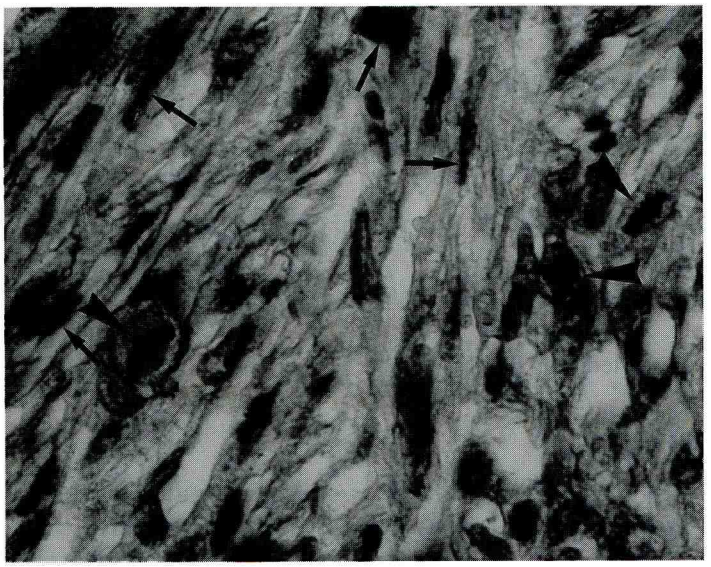

Figure 1. The cardiac leiomyosarcoma immunostained with anti-smooth muscle cell actin antibody. Bundles of actin filaments are readily seen (arrows). Mitoses (arrowheads). Magnification x400.

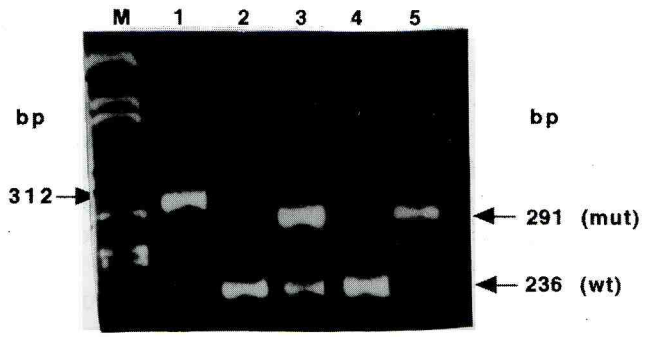

Figure 2. Detection of H-*ras* codon 12 mutations by PCR-RFLP analysis. PCR products were digested with MspI restriction endonuclease and electrophoresed through a 6% agarose gel. Lane 1, undigested PCR product (312 bp), lanes 2 and 4, negative samples (236 bp); lane 3, positive sample (291 bp); lane 5, positive control (SW 480 cell line) M, Marker pUC18/HaeIII. mut, mutant; wt, wild-type.

Primary cardiac malignant neoplasms are very rare and the prognosis of patients with such tumors remains poor (8). Surgical resection of these neoplasms followed by adjuvant therapy, appears to have improved survival in patients with primary cardiac malignancies (9). The implication of *ras* family genes in the development of primary cardiac malignancies has not been previously reported in patients with such neoplasms. Review of the literature showed that some cases of primary cardiac leiomyosarcomas have been described in recent years, which have emphasized the clinical presentation and the prognosis of leiomyosarcoma patients (8,9). To our knowledge, this is the first report of a primary cardiac leiomyosarcoma studied by molecular biology techniques for *ras* family gene mutations. Our molecular analysis of the cardiac leiomyosarcoma tissue specimen, showed the presence of a point mutation at codon 12 of H-*ras* gene.

This observation indicates that the H-*ras* gene may have a role in the development of primary cardiac leiomyosarcoma. A more detailed analysis involving a large number of patients is required in order to establish the precise role of the *ras* family genes in primary cardiac malignancies. Further studies using molecular biology techniques may elucidate the pathogenesis of these rare neoplasms.

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