Human Pathology (2017) **60**, 199–207

ELSEVIER

Human PATHOLOGY

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Original contribution

Cardiac angiosarcoma: histopathologic, immunohistochemical, and cytogenetic analysis of 10 cases☆

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Received 20 August 2016; revised 20 September 2016; accepted 14 October 2016

Keywords:
Angiosarcoma;
Cardiac tumors;
Cytogenetics;
Molecular diagnostics;
Cardiac malignancy

Summary Angiosarcoma (AS) is the most common cardiac sarcoma with differentiation, and is poorly characterized from a molecular genetic standpoint. Prognosis remains poor, owing to several factors including aggressive tumor biology, poor response to adjuvant therapy, and lack of targeted therapy. The clinical, pathologic and molecular cytogenetic features were studied in ten cardiac AS surgically resected at Mayo Clinic (1994–2015) using a whole-genome, single-nucleotide polymorphism-based platform (OncoScan). Mean patient age was 47.8 years, male/female ratio was 1:1.5, and overall median survival was 5.2 months. The most common location was the right atrium (n = 7), with one case each occurring in the epicardium, pericardium, and right ventricle. No patients had received thoracic irradiation. The most common morphology was spindle cell (n = 8), with one case each of epithelioid and biphasic. ERG was the most sensitive vascular marker, with diffuse immunoreactivity in all cases. Several recurrent (present in at least 3 cases) aberrations were identified, including trisomies in chromosomes 4, 8, 11, 17, 20, as well as 1q+, and homozygous deletion of *CDKN2*. Patients who received adjuvant therapy had longer overall survival than those who did not (median 13.4 *vs* 3.2 months; $P = .0283$). There were no significant associations between tumor location, histology, immunohistochemical findings, cytogenetic profile, and clinical outcome; however, there was a trend towards improved overall survival in patients with tumors harboring 1q+ (median 31.8 *vs* 3.7 months, $P = .06$). This study confirms recurrent cytogenetic aberrations in cardiac AS, some of which may have prognostic or predictive implications.

1. Introduction

Angiosarcoma (AS) is a rare malignant vascular neoplasm, representing approximately 1% of all sarcomas [1]. It occurs most commonly in the skin of the head and neck, breast, and in deep soft tissues, and rarely in visceral locations such as the liver and spleen [2]. While the heart is an uncommon site, AS is somewhat overrepresented due to the relative rarity of

☆ Disclosures: The authors have no conflicts of interest or funding to disclose.

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http://dx.doi.org/10.1016/j.humpath.2016.10.014
0046-8177/

primary cardiac malignancies. As such, AS represents approximately 40% of all cardiac sarcomas, and is thus the most common differentiated cardiac sarcoma [3,4]. Prognosis in cardiac AS is poor, with median overall survival ranging from 5 to 13 months, owing to several factors including aggressive tumor biology, the challenging nature of surgical resection, poor response to adjuvant therapy, and the lack of targeted therapy [4-6].

As a group, primary cardiac AS remains poorly characterized from an etiologic and molecular genetic standpoint. Known risk factors for cutaneous and visceral AS, such as ionizing radiation and long-term exposure to synthetic material, have each been documented in a single case of cardiac AS [7,8]. However, most cases remain idiopathic. There appears to be some basis for a genetic predisposition, based on the clustering of cardiac AS within certain families [9,10]. In addition, somatic point mutations in *KRAS*, *TP53*, and *PLCG1* have been identified in some cases of cardiac AS, as have large-scale genomic gains and losses, some of which appear to be recurrent in a subset of cases [11,12]. However, owing to the rarity of cardiac AS, reports to date have consisted of case reports and small case series, which have limited the identification of recurring pathogenetically, and/or prognostically significant, molecular aberrations.

This study aims to further characterize the molecular genetic features of primary cardiac AS, and to correlate such with histopathologic and clinical features. In particular, we sought to identify recurrent cytogenetic aberrations that may serve as prognostic markers as well as guide identification of therapeutic targets.

2. Materials and methods

2.1. Case selection

Tissue registry archives at Mayo Clinic (Rochester, MN) were queried for cases of cardiac AS (1995–2014). Cases with sufficient tumor content (to reach target of 100 ng DNA) for molecular genetic testing were retained for inclusion in the study cohort. Patient demographics and clinical follow-up and outcome were abstracted from the patient record. The study was approved by the Mayo Clinic Institutional Review Board and Biospecimens Subcommittee.

2.2. Gross and histopathologic features

Tumor location and size were abstracted from the patient record (including radiology, surgery, and pathology reports). Hematoxylin and eosin (H&E)–stained tissue sections of the tumor were reviewed, and the following features were assessed: cytomorphology (spindle cell, epithelioid, or biphasic); tumor necrosis (present or absent); mitotic activity (number of mitotic figures per 10 high-power fields [HPF, 400×] averaged over 30 HPFs).

2.3. Immunohistochemistry

Immunoperoxidase studies were carried out on formalin-fixed, paraffin-embedded (FFPE) tissue sections (4-μm thick) with the following antibodies: Broad-spectrum cytokeratin (Dako, Santa Clara, CA; clones AE1 and AE3; 1:200); CD34 (Leica, Buffalo Grove, IL, clone QBEnd/10, 1:50); CD31 (Dako, clone JC/70a, 1:350); ERG (Biocare, Concord, CA, clone 9FY, 1:25); and FLI1 (Pharmingen, San Jose, CA, clone G146–254 BD, 1:50). Tumor reactivity for immunohistochemical antibodies was scored based on distribution (diffuse, >50% tumor cells; focal, <50% tumor cells), and intensity (0, negative; 1+, weak reactivity; 2+, moderate reactivity; 3+, strong reactivity).

2.4. Molecular genetic analysis

DNA was extracted from 5-μm-thick scrolls prepared from FFPE tissue block. DNA was extracted using QIAamp DNA FFPE Tissue Kit (QIAGEN, Germantown, MD) following the Affymetrix recommended protocol (Affymetrix P/N 703175 Rev. 2, Appendix E). Extracted DNA was quantified using a Qubit 2.0 Fluorometer (Life Technologies/Thermo Fisher Scientific, Waltham, MA) and the dsDNA BR assay kit according to the manufacturer's protocol. This was done in triplicate to provide each of the three test laboratories with enough DNA to run the assays. The Affymetrix OncoScan assay was then performed per the manufacturer's protocol; this whole-genome, single-nucleotide polymorphism–based microarray platform assesses copy number and loss of heterozygosity. The OncoScan assay utilizes molecular inversion probe (MIP) technology, for the identification of copy number alterations and loss of heterozygosity (LOH). MIP probes in the OncoScan assay capture the alleles of over 220 000 SNPs at carefully selected genomic locations, distributed across the genome with increased probe density within ~900 cancer genes.

Array fluorescence intensity data (CEL files), generated by Affymetrix GeneChip Command Console (AGCC) Software version 4.0, were processed using OncoScan Console software version 1.1.034 to produce OSCHP files. After conversion of CEL files by OncoScan Console, OSCHP files were loaded into the Chromosome Analysis Suite (ChAS) software version 3.1 for analysis of clinically relevant CN and LOH events. All regions flagged by the ChAS software were investigated. Autosomal probes were considered "copy number loss" if the genomic interval deviating from normal contained a minimum of 25 probes. Autosomal probes were considered "copy number gain" if the genomic interval deviating from normal contained a minimum of 50 probes. Absence of heterozygosity (AOH) calls were made when a region of AOH was greater than 5 mb. Any regions not flagged by the software but found to deviate from normal were investigated and included in the list of final calls as appropriate.

2.5. Statistical analysis

Overall survival was estimated with the Kaplan–Meier method, and was summarized with the median survival and