Human Pathology (2015) **46**, 690–697

ELSEVIER

Human PATHOLOGY

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Original contribution

Cutaneous basal cell carcinosarcomas: evidence of clonality and recurrent chromosomal losses

Paul W. Harms MD, PhD[a,b,c,*], Douglas R. Fullen MD[a,b], Rajiv M. Patel MD[a,b], Dannie Chang MD[b], Sara C. Shalin MD, PhD[d], Linglei Ma MD, PhD[e], Benjamin Wood BMed, FRCPA[f], Trevor W. Beer MBChB, FRCPath[g], Javed Siddiqui MS[a,c], Shannon Carskadon MS[h], Min Wang PhD[a], Nallasivam Palanisamy MSc, MPhil, PhD[a,c,h,i], Gary J. Fisher PhD[b], Aleodor Andea MD[a,b]

[a]*Department of Pathology, University of Michigan Health System, Ann Arbor MI 48109*
[b]*Department of Dermatology, University of Michigan Health System, Ann Arbor MI 48109*
[c]*Michigan Center for Translational Pathology, University of Michigan Health System, Ann Arbor MI 48109*
[d]*Departments of Pathology and Dermatology, University of Arkansas for Medical Sciences, Little Rock, AR 72205*
[e]*Miraca Life Sciences, Glen Burnie, MD 21061*
[f]*School of Pathology and Laboratory Medicine, University of Western Australia, Perth, WA 6009, Australia*
[g]*CliniPath Pathology, Osborne Park, WA 6017, Australia*
[h]*Department of Urology, Henry Ford Health System, Detroit, MI 48202*
[i]*King Saud University, Riyadh, Saudi Arabia 11362*

Received 21 November 2014; accepted 3 January 2015

Keywords:
Carcinosarcoma;
Osteosarcoma;
Basal cell carcinoma;
Clonality;
Copy number analysis;
TP53

Summary Cutaneous carcinosarcomas are heterogeneous group of tumors composed of malignant epithelial and mesenchymal components. Although mutation analyses have identified clonal changes between these morphologically disparate components in some subtypes of cutaneous carcinosarcoma, few cases have been analyzed thus far. To our knowledge, copy number variations (CNVs) and copy-neutral loss of heterozygosity (CN-LOH) have not been investigated in cutaneous carcinosarcomas. We analyzed 4 carcinosarcomas with basal cell carcinoma and osteosarcomatous components for CNVs/CN-LOH by comparative genomic hybridization/single-nucleotide polymorphism array, *TP53* hot spot mutations by polymerase chain reaction and Sanger sequencing, and *TP53* genomic rearrangements by fluorescence in situ hybridization. All tumors displayed multiple CNV/CN-LOH events (median, 7.5 per tumor). Three of 4 tumors displayed similar CNV/CN-LOH patterns between the epithelial and mesenchymal components within each tumor, supporting a common clonal origin. Recurrent changes included allelic loss at 9p21 (*CDKN2A*), 9q (*PTCH1*), and 17p (*TP53*). Allelic losses of chromosome 16 including *CDH1* (E-cadherin) were present in 2 tumors and were restricted to the sarcomatous component. *TP53* mutation analysis revealed an R248L mutation in both epithelial and mesenchymal components of 1 tumor. No *TP53* rearrangements were identified. Our findings indicate that basal cell carcinosarcomas harbor CNV/CN-LOH changes similar to conventional basal cell carcinoma, with

* Corresponding author. 3261 Medical Science I, 1301 Catherine, Ann Arbor, MI 48109–5602.
E-mail address: harmsa2fam@gmail.com (P. W. Harms).

http://dx.doi.org/10.1016/j.humpath.2015.01.006
0046-8177/Published by Elsevier Inc.

additional changes including recurrent 9p21 losses and a relatively high burden of copy number changes. In addition, most cutaneous carcinosarcomas show evidence of clonality between epithelial and mesenchymal components.
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1. Introduction

Carcinosarcomas are biphasic tumors composed of malignant epithelial and mesenchymal components. Cutaneous carcinosarcomas typically arise in sun-exposed skin of the elderly [1-3]. The epithelial component of cutaneous carcinosarcomas most commonly resembles basal cell carcinoma (BCC) or squamous cell carcinoma and, less frequently, adnexal carcinomas or Merkel cell carcinoma. The mesenchymal component may be undifferentiated or display osteosarcomatous, chondrosarcomatous, leiomyosarcomatous, rhabdomyosarcomatous, or fibrosarcomatous differentiation [3].

The most common form of cutaneous carcinosarcoma with heterologous differentiation is basal cell carcinosarcoma with an osteosarcomatous mesenchymal component [1-11]. Rigorous analysis of clinical outcomes has been challenging given the rarity of these tumors. Excision is curative in many cases. However, metastasis and death have occurred among the relatively small number of reported cases of basal cell carcinosarcoma [3], suggesting that these tumors may follow a more aggressive course than BCC.

Cancers arise in association with genetic events including point mutations, insertions/deletions, chromosomal rearrangements, copy number variation (CNV), and copy-neutral loss of heterozygosity (CN-LOH) [12]. Most conventional BCCs display recurrent inactivating mutations in tumor suppressors *PTCH1* and/or *TP53* and harbor a low number of CNV/CN-LOH events [13-16]. In contrast, a true osteosarcoma is a karyotypically complex malignancy; however, it also often displays *TP53* rearrangements [17,18]. Similar to BCC, basal cell carcinosarcomas may harbor inactivating mutations of *TP53* or *PTCH1* [11,19-21].

A fundamental question regarding the histogenesis of cutaneous carcinosarcomas is whether the carcinomatous and sarcomatous components are clonally related [3,22]. Most carcinosarcomas at other sites display evidence of monoclonal origin [22], and previous mutation analyses in 3 basal cell carcinosarcomas demonstrated evidence of monoclonal origin [19-21]. None of the basal cell carcinosarcoma cases previously analyzed displayed heterologous osteosarcomatous differentiation.

Because oncogene activation and tumor suppressor inactivation may occur by copy number change rather than mutation [12], copy number profiling is essential for comprehensive understanding of genetic changes in a malignancy. To better understand copy number profiles and clonal relatedness in basal cell carcinosarcomas, we assembled a cohort of 5 basal cell carcinosarcomas with osteosarcomatous components, 4 of which were suitable for molecular analysis.

2. Materials and methods

2.1. Case review

All studies were conducted in accordance with protocols approved by the University of Michigan Institutional Review Board. Five cases of basal cell carcinosarcoma with osteosarcomatous component, which provided DNA suitable for polymerase chain reaction (PCR), were obtained for this study. One case was excluded because of DNA unsuitable for PCR. Three of the remaining 4 cases were previously reported in the literature without molecular analysis (Table 1). The fourth case was obtained from Miraca Life Sciences. All cases were reviewed by P.W.H. and A.A. for diagnostic confirmation and to evaluate suitability for laser capture microdissection. For all cases, areas with a minimum of 65% purity for the sarcomatous component and 70% purity for the carcinomatous component were identified, confirmed by hematoxylin and eosin (H&E) and cytokeratin staining (Supplementary Table S1).

2.2. Immunohistochemistry

Immunohistochemistry for p63 and vimentin was performed on a Ventana Benchmark automated stainer (Ventana Medical Systems, Tucson, AZ) using mouse monoclonal anti-p63 antibody (clone 4A4, predilute; Ventana Medical Systems) or mouse monoclonal anti-vimentin antibody (clone V9, predilute; Ventana Medical Systems) as previously described [23]. Immunohistochemistry for K903 was performed on a Dako automated stainer using 1:100 dilution

Table 1 Clinicopathological features and prior published descriptions of basal cell carcinosarcomas analyzed in this study

Case	Age (y)	Sex	Site	Reference
1	87	M	Ear	Harvey et al 2014 [3] (case 1)
2	>90	F	Arm	Elwood et al 2014 [2]
3	73	M	Calf	Harvey et al 2014 [3] (case 4)
4	69	M	Calf	No prior report

Abbreviations: M, male; F, female.