Author's response to reviews

Title: Blastomatoid pulmonary carcinosarcoma: report of a case with a review of the literature

Authors:

Inga-Marie Schaefer (schaeferinga@web.de)
Carsten-Oliver Sahlmann (csahlmann@med.uni-goettingen.de)
Tobias Overbeck (overbeck@med.uni-goettingen.de)
Stefan Schweyer (s.schweyer@pathologie-starnberg.de)
Jan Menke (menke-j@t-online.de)

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Author's response to reviews: see over
Letter of revision

Dear Christna Chap,

First of all I would like to thank you for giving us the opportunity to submit a revised version of our manuscript for possible publication in BMC Cancer and for your helpful comments. Please find below our detailed responses to the reviewers' comments:

To Reviewer 1: Florian Laenger

Major Compulsory Revisions:

1) rather extensive molecular work has been already done, CGH and/or CISH data of the two distinct components of the tumor would be very interesting regarding molecular tumorigenesis and possibly improve the diagnostic value of the molecular data for small biopsies

- We agree that it would indeed be very interesting to examine both tumor components separately, in particular to assess the presence of (differential) chromosomal imbalances by CGH. However, the two components were closely intermixed as demonstrated in Figure 2, and the epithelial elements consisted of only small glands surrounded by the stromal component. Therefore, it was not possible to separate both components under the light microscope. Also by using laser microdissection it is, from our personal experience, not possible to render DNA that is in regard of quality and amount sufficient enough to perform reliable CGH. As demonstrated previously for pulmonary carcinosarcoma (Dacic et al.), biphasic pulmonary blastoma (Takahashi et al.), and carcinosarcomas of other localization (Thompson et al.), both components are believed to be monoclonal in origin. We therefore
added “The developmental origin of both tumor components is unclear and an origin from two or more stem cells (multiclonal hypothesis) or an origin from a single totipotential stem cell that differentiates into separate epithelial and mesenchymal directions (monoclonal hypothesis) seems possible [14]. Previous analyses in pulmonary carcinosarcoma [15], biphasic pulmonary blastoma [12], and carcinosarcomas of other localizations [14] provide evidence that the epithelial and mesenchymal component of these biphasic tumors harbor a different morphology, but are monoclonal in origin” in the discussion on page 7.

2) histological description of the tumor does not accurately mention the most salient diagnostic features of pulmonary carcinosarcomas regarding both components and is sometimes misleading: nuclear atypia per se does not indicate high grade fetal adenocarcinoma as indicated in the text, but the branching (immature) architecture of glands (fig. 2A) which are composed of columnar cells with palisading elliptic nuclei with subnuclear vacuoles (fig. 2B)

• As proposed, we added the morphologic feature of “a branching (immature) architecture of glands composed of columnar cells with palisading elliptic nuclei with subnuclear vacuoles” of the glandular component to the case presentation on page 4, and “branching glands composed of columnar cells with palisading elliptic nuclei with subnuclear vacuoles” in the discussion on page 6.

Minor essential revisions:

1) of mesenchymal tumor cells expressing desmin and myoD1

• We specified the percentage of mesenchymal tumor cells expressing desmin (“in 10% of tumor cells”) on page 4. In the meantime we established myogenin as a more sensitive myogenic marker. Therefore, we replaced the mentioning of MyoD1 by myogenin in the text on page 4 and in Table 1.

2) morules in blastomas are of epithelial origin and differentiation, thus they should not be mentioned in the context of mesenchymal components as this might be misleading

• We instead mentioned the morules in the context of the epithelial component on page 6.

3) check wording and language, e.g. “expression …. was not expressed”

• We corrected the expression to “expression…was not observed” on page 6.
4) I personally would discourage statements as “TP53 mutation was detected immunohistochemically”, as IH is a rather unspecific surrogate marker for mutations in this context.
   - As proposed, we refrained from the expression “TP53 mutation was detected immunohistochemically” and replaced it by “Immunohistochemically, an expression of p53 was detected in the present case consistent with an underlying TP53 mutation…” on page 7.

5) There are divergent statements regarding the gain of chr 12 (12q14q21/12q24 and 12q13q21/12q12q21
   - The amplification at chromosome was detected at 12q14q21, as described. However, on page 8 we mentioned “The amplicon 12q13q21 (including the observed 12q14q21) is also typically detected in different types of sarcoma, particularly in liposarcoma and osteosarcoma” to explain to the reader that the previously reported region 12q13q21 also includes the here observed amplified region 12q14q21.

6) Analysis of SYT-SSX fusion gene is mentioned, but the reason for this analysis, which is to exclude synovial sarcoma (as another biphasic tumor) is omitted
   - As proposed, we added an explanation for why the analysis of SYT-SSX fusion gene was performed in the case presentation on page 4 (“A SYT-SSX fusion gene suggestive of synovial sarcoma was not detected by RT-PCR”)

7) Other potential differential diagnoses are not mentioned, e.g. synovial sarcoma, teratoma etc.
   - As suggested, we added and discussed the differential diagnoses on page 5 (“The differential diagnoses of biphasic pleuropulmonary tumors in adults include glandular malignant peripheral nerve sheath tumor (MPNST), synovial sarcoma, and malignant mesothelioma [2,3,5]. When only small biopsy specimen are available and both components are not represented, carcinosarcoma may also be misinterpreted as an either entirely epithelial or mesenchymal neoplasms [2]. In glandular MPNST, rhabdomyosarcomatous elements may be present, but the tumor usually displays intestinal type-epithelium with goblet cells. Furthermore, the sarcomatoid part of MPNST expresses S100 protein and vimentin, and the tumor is associated with neurofibromatosis type 1 [6]. Synovial sarcoma was ruled out because the characteristic SYT-SSX fusion gene was not detected [7]. Malignant mesothelioma was also ruled out by negative staining for mesothelial markers (i.e. calretinin, D2-40). Furthermore, pulmonary blastoma should be
considered in the differential diagnosis if the epithelial component consists of adenocarcinoma [2]).

Discretionary Revisions

1) the characteristics of the blastomatoid variant pulmonary carcinosarcoma in comparison to conventional carcinosarcoma are given in table 1, they should be highlighted in the text also, esp. as this variant is not officially recognized in the current WHO classification

- As proposed, we added the characteristics of the blastomatoid variant pulmonary carcinosarcoma in comparison to conventional carcinosarcoma to the text on page 5 along with citation of the current WHO classification of tumours (“The blastomatoid variant of carcinosarcoma is not yet recognized as a distinct entity by the WHO classification of tumours [3]. In contrast to conventional pulmonary carcinosarcoma, which contains squamous cell carcinoma, adenocarcinoma, adenosquamous carcinoma, large cell carcinoma as epithelial component, the blastomatoid variant of carcinosarcoma comprises high-grade adenocarcinoma of the fetal lung type/clear cell adenocarcinoma with fetal lung features [1]. This is a typical feature of pulmonary blastoma and therefore led to the designation as "blastomatoid pulmonary carcinosarcoma [1]". Accordingly, Table 1 was corrected and rearranged to highlight that the blastomatoid carcinosarcoma is a variant of conventional pulmonary carcinosarcoma.

To Reviewer 1: Ryu Kanzaki

Major Compulsory Revisions:

1) The reviewer thinks that discussion on whether adjuvant chemotherapy should be administered in this case is needed.

- We added “The epithelial component, accounting for ~40% of the vital tumour…” on page 4-5 to specify the ratio of the mesenchymal and epithelial component of the tumour. Since the sarcomatous elements slightly predominated, adjuvant therapy according to the guidelines for soft tissue sarcomas was discussed in this individual case, but the patient refused. We added “An optional adjuvant therapy was discussed with the patient, but he refused” on page 4, and “As reported, complete surgical resection is the treatment of choice in patients with resectable tumors [2]. Chemotherapy and radiation can be used in an adjuvant setting although specific regimens do not exist [2,3]. In the present case, the
mesenchymal component slightly predominated and an adjuvant therapy according to guidelines for soft tissue sarcomas was discussed. However, since complete resection was achieved, lymph node or distant metastases were ruled out, and the patient refused, no adjuvant therapy was applied” on page 8.

We very much hope that the revised version of our manuscript now complies with the journal's requirements for publication.

Sincerely

Inga-Marie Schaefer, MD
Corresponding author