Histopathology

Histopathology 2023, **82**, 576–586. DOI: 10.1111/his.14840

Salivary carcinosarcoma: insight into multistep pathogenesis indicates uniform origin as sarcomatoid variant of carcinoma ex pleomorphic adenoma with frequent heterologous elements

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Date of submission 25 August 2022
Accepted for publication 5 November 2022
Published online *Article Accepted* 14 November 2022

Ihrler S, Stiefel D, Jurmeister P, Sandison A, Chaston N, Laco J, Zidar N, Brcic L, Stoehr R & Agaimy A (2023) *Histopathology* **82**, 576–586. https://doi.org/10.1111/his.14840

Salivary carcinosarcoma: insight into multistep pathogenesis indicates uniform origin as sarcomatoid variant of carcinoma ex pleomorphic adenoma with frequent heterologous elements

Aims: The formal pathogenesis of salivary carcinosarcoma (SCS) remained unclear, both with respect to the hypothetical development from either preexisting pleomorphic adenoma (PA) or *de novo* and the clonal relationship between highly heterogeneous carcinomatous and sarcomatous components.

Methods and results: We performed clinicopathological and molecular (targeted RNA sequencing) analyses on a large series of 16 cases and combined this with a comprehensive literature search (111 cases). Extensive sampling (average 11.6 blocks), combined with immunohistochemistry and molecular studies (PA-specific translocations including *PLAG1* or *HMGA2* proven in 6/16 cases), enabled the morphogenetic identification of PA in 15/16 cases (93.8%), by far surpassing a reported rate of 49.6%. Furthermore, we demonstrated a multistep (intraductal/intracapsular/extracapsular) adenoma-carcinoma-sarcoma-progression, based on two alternative histogenetic pathways (intraductal, 56.3%, versus myoepithelial pathway, 37.5%). Thereby, early intracapsular stages are identical to conventional carcinoma ex PA, while later extracapsular stages are dominated by secondary, frequently heterologous sarcomatous transformation with often large tumour size (>60 mm).

Conclusion: Our findings strongly indicate that SCS (almost) always develops from PA, with a complex multistep adenoma-carcinoma-sarcoma-sequence, based on two alternative histogenetic pathways. The findings from this novel approach strongly suggest that SCS pathogenetically is a rare (3–6%), unique, and aggressive variant of carcinoma ex PA with secondary sarcomatous overgrowth. In analogy to changes of terminology in other organs, the term "sarcomatoid carcinoma ex PA with/without heterologous elements" might be more appropriate.

Keywords: carcinosarcoma, gene fusion, HMGA2, molecular testing, PLAG1, pleomorphic adenoma, salivary glands, true malignant mixed tumour

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Introduction

Salivary carcinosarcoma (SCS) is a rare aggressive malignancy, characterised by a biphasic, highly variable combination of malignant epithelial and (frequently heterologous) mesenchymal components,[1–5] manifesting in major and, rarely, minor glands. Analogous to the historic term "benign mixed tumour" (now pleomorphic adenoma, PA), this tumour type was initially called "true malignant mixed tumour", with, at that time, a blurred distinction from carcinoma ex pleomorphic adenoma (CEPA).[1,2,6–10] Later, SCS became the universally accepted terminology[3–5,8,11–14] and remained so in the current 4th[11] and upcoming 5th[15] WHO classification of salivary tumours. The distinction of SCS from high-grade sarcomatoid dedifferentiation of primary salivary carcinoma (mostly salivary duct carcinoma, SDC) has not been clearly defined.[8,13,16,17] SCS typically presents as large, infiltrative, histologically high-grade, and aggressive tumours. To date, 111 cases have been published, the largest series comprising 12 cases.[1,11]

The pathogenesis of SCS is poorly understood. It has been hypothesised that SCS may develop either from preexisting PA or *de novo*. An associated PA has been described in half of the cases.[1,3–5,8–12] However, this figure may be too low, as the frequency of PA detection is strongly influenced by awareness of this association, the extent of tissue sampling, and the fact that sclerosed PA can be misinterpreted as regressive changes. In addition, the relationship between carcinomatous and sarcomatous components has been disputed (as in carcinosarcoma of other organs[18]). A clonal relationship between both components has been proven,[14,18] and derivation of the sarcomatous component has been postulated from myoepithelial cells of PA and from primary carcinomatous components.[1,4,8–10,12]

Concerning CEPA, a complex multistep carcinogenesis has been characterised. With the exception of the myoepithelial type, intracapsular CEPA generally starts as intraductal neoplasia within the biphasic tubules of PA. While intraductal and intracapsular-invasive stages are associated with favourable prognosis, extracapsular invasion exhibits impaired prognosis. Thereby, minor invasion (≤6 mm[11]) is associated with moderately increased and extensive invasion with significantly increased tumour progression and death from disease.[19–25] The present study is a follow-up of our previous studies,[19,20,26] where five SCS ex PA were identified among 85 CEPA cases (5.9%).[20]

The 16 cases represent the largest SCS series published to date. We performed clinical, (immuno-) histological, and molecular investigations as previously described for CEPA.[19,20,26] As gene fusions including *PLAG1* and *HMGA2* have been identified as context-specific for PA,[13,27,28] we applied targeted RNA sequencing looking for PA-typical fusions as a surrogate for preexisting PA, missed on histological sampling.

This study aimed to address a better understanding of the pathogenesis of SCS with the following key questions: (i) How frequently does SCS develop from PA, or alternatively, do *de novo* SCS really exist? (ii) What is the pathogenetic relationship between carcinomatous and sarcomatous components? (iii) Can SCS be regarded as a special variant of CEPA? To answer these questions, we performed a combined analysis of our series and a comprehensive literature search.

Materials and Methods

ANALYSIS OF OUR CASE SERIES

Cases were retrieved from consultation files of one author (S.I.) and from six European centres from 1999 to 2019. Five cases were part of a previous study on CEPA.[20] All cases were reviewed by two authors (S.I., A.A.). All available blocks (2–39; average 11.6/ per case), were screened using haematoxylin and eosin (HE-)stains for PA, and in areas suspicious for PA, appropriate immunohistochemical reactions were applied.[19,20] Clinical information was searched for longstanding preoperative tumours (typically PA) and for previous surgical procedures.

The analysis took into account well-established intraductal, intracapsular, and extracapsular-invasive progression stages, as described for CEPA.[19–21,23–26] Immunohistochemical staining for CK14, CK18, p63, SOX10, Ki67, p53, Her2-neu, and androgen receptor (AR) was generally performed (details of antibodies, clones, antigen retrieval, controls, and scoring, as described previously[20]). Additional staining (actin, desmin, myogenin, MyoD1, S100, CD31, CD34, ERG) was facultatively applied. Cases of high-grade, typically spindle-cell ("sarcomatoid") dedifferentiation of classical salivary carcinoma types, devoid of heterologous differentiation, were excluded.[16,17] Carcinomatous and sarcomatous components were recorded semiquantitatively, and histological grade were evaluated for intra−/extracapsular tumour. The presence of heterologous sarcomatous components and extent of extracapsular invasion were documented.[19,20] Sarcomatous components were subtyped using criteria as for soft-tissue tumours.

NEXT-GENERATION SEQUENCING

Next-generation sequencing (NGS) was performed using RNA isolated from formalin-fixed, paraffin-embedded tumour tissue sections with the TruSight RNA Fusion Panel (Illumina, San Diego, CA, USA) as previously described.[28] The Integrative Genomics Viewer (IGV), version 2.13.3 (Broad Institute, https://software.broadinstitute.org/software/igv/) was used for data visualisation.[29]

SEARCH OF THE LITERATURE

A systematic search of the English literature was performed using PubMed and Google Scholar databases, applying the program Endnote (Version X.9.3.3.). A combination of these keywords was applied: salivary gland, carcinosarcoma, (true) malignant mixed tumour, and carcinoma ex PA. All relevant clinical, histomorphological, and molecular data were extracted.

Results

FINDINGS FROM CASE SERIES

Detailed clinical and histomorphological data are presented in Table 1. There were seven men (43.8%) and nine women (56.2%), aged 22–86 years (median 65.8); 13 tumours were in parotid (81.2%), one in submandibular (6.3%), and two in minor glands (12.5%). A PA component could be verified histomorphologically (or immunohistochemically if needed), in 13 cases (1–13; Figure 1). The size of PA ranged from 13–60 mm (average, 21). PA manifested as multinodular recurrence in two cases.

Intracapsular malignant tumour within PA was present in 12 patients (no. 1–12). Intraductal carcinoma was present in 9/12 (no. 1–9, all high-grade, Figures 1, 2B), intracapsular-invasive carcinoma in 11 (frequently undifferentiated and salivary duct carcinoma, n = 5 each), and intracapsular sarcoma in eight cases (frequently chondrosarcoma, n = 6). Concerning intracapsular tumour, carcinomatous components quantitatively dominated over sarcomatous components (the latter absent in four cases). Histological grade was poorly differentiated in 11 and moderately in one case.

Extracapsular tumour displayed variable combinations of carcinomatous and sarcomatous components in 12 (Figures 1, 2) and pure sarcomatous components in three cases. Most frequent carcinoma types were SDC, undifferentiated, and myoepithelial carcinoma (n = 4 each), most frequent sarcoma pattern was chondro-, undifferentiated pleomorphic (n = 7 each), and osteosarcoma (n = 3). Extracapsular sarcomatous components were mostly large, frequently with extensive necrosis, all poorly differentiated, and quantitatively dominated the smaller carcinomatous components.

Combining intra- and extracapsular tumours, there were two malignant components (one carcinoma and one sarcoma type each) in 43.7%, three in 37.5%, and four and five in 6.3% each. Heterologous sarcomatous differentiation manifested in 13 cases (81.3%).

In nine cases with intraductal carcinoma, there was immunohistochemical overexpression for p53 in six, for AR in seven, and for Her2neu in three cases (Figure 1). Concerning extracapsular malignant components, seven cases each showed overexpression of p53 and AR and one overexpressed Her2neu. Comparing intraductal carcinoma and extracapsular malignant tumour, there was concordance in six and discordance in three cases each with regard to overexpression/negativity for p53 and AR. Comparing carcinomatous and sarcomatous components, there was concordance in nine and discordance in one case concerning overexpression/negativity for p53, and concordance and discordance in six cases each, concerning AR.

Targeted RNA sequencing was successful in 11 patients. Gene fusions were detected in eight cases. *PLAG1* fusion was the most common (5/8). *CTNNB1* (n = 2), *FGFR1* (n = 2), and *HNRNPA2B1* (n = 1) were *PLAG1* fusion partners. One tumour had *HMGA2::WIF1* fusion. Another had a novel *YWHAE::GNB1* fusion verified using a *YWHAE* break-apart (Fluorescence In Situ Hybridization) FISH probe. Without functional analyses, this fusion remains of unknown significance. Another case revealed a *KDM5A::CACNA1C* fusion of unknown significance. Of six cases with PA-typical translocations, four showed concomitant histomorphological evidence, and two lacked evidence of PA, indicating that PA was either overgrown by SCS or missed on sampling (no. 14,15). Combining the histomorphological and molecular approach, a preexisting PA could be proven in 15/16 cases (93.8%; Table 1). Of nine cases with matched pairs of intra−/extracapsular components, concordant positive (n = 5) and negative (n = 3) molecular results were observed, consistent with common clonal origin. One tumour (no. 15) had *PLAG1::FGFR1* fusion in the intracapsular, not detected in the extracapsular component.

Table 1. Clinical, histomorphological, and molecular data in case series

Clinical data					Pleomorphic adenoma		Intracapsular malignant component			Extracapsular malignant component	
Nr	Sex	Age	Site	pTN	Histol	NGS	ID	Invasive carcinoma	Sarcoma	Carcinoma	Sarcoma
1	M	56	P	T4, Nx	Yes	YWFAE/GNB1	**ID**	**sal duct**	**ø**	sal duct	**pleomorph** chondro
2	M	46	P	T2, N0	Yes	n. e.	**ID**	**sal duct**	**ø**	sal duct	**pleomorph**
3	M	51	P	T4, N2	Yes	No fusion	**ID**	undiff sal duct	**pleomorph osteo** chondro	undiff sal duct	**osteo** pleomorph
4	F	50	P	T2, N0	Yes	n. e.	**ID**	squamous	**osteo chondro**	undiff	**osteo**
5	F	85	P	T3, N1	Yes	n. e.	**ID**	undiff	**pleomorph**	**ø**	**pleomorph**
6	M	78	P(d)	T4, N2	Yes	KDM5A/CACNA1C	**ID**	undiff	**chondro**	undiff	**osteo**
7	F	64	P	T3, Nx	Yes	CTNNB1/PLAG1	**ID**	sal duct undiff	**myogenic**	**ø**	**myogenic**
8	F	83	P	T3, N0	Yes*	CTNNB1/PLAG1	**ID**	undiff	**ø**	undiff	**chondro**
9	F	81	P	T2, N0	Yes	n. e.	**ID**	**sal duct**	chondro	sal duct	**chondro**
10	F	59	M	T4, Nx	Yes	HMGA2/WIF1	**ø**	acc	**ø**	acc	**spindle cell** pleomorph
11	M	57	S	T4, N2	yes	No fusion	**ø**	**myoep**	chondro	**ø**	**pleomorph**
12	F	22	P	T1, Nx	Yes	HNRNPA2B1-PLAG1	**ø**	**ø**	**chondro**	**ø**	**ø**
13	F	72	M	T2, Nx	Yes*	n. e.	-	-	-	myoep	**chondro**
14	F	85	P	T3, N0	No	FGFR1/PLAG1	-	-	-	myoep	**pleomorph** chondro
15	M	86	P	T4, Nx	No	FGFR1/PLAG1	-	-	-	myoep	**chondro**
16	M	78	P	T4, N3	No	No fusion	-	-	-	myoep squamous	**chondro**

Bold letters: Dominant malignant tumour component.
Blue colour: Histological + molecular evidence of PA.
Yellow colour: Intraductal carcinoma pathway.
Grey colour: myoepithelial carcinoma pathway.
*, Recurrent PA; −, PA absent; acc, Adenoidcystic carcinoma; chondro, Chondrosarcoma; F, female; histol, histological; ID, Intraductal carcinoma; M, male; M, Minor salivary gland; myoep, Myoepithelial carcinoma; myogenic, Myogenic sarcoma; n.e., not evaluable; NGS, Next-generation sequencing; ø, component absent; osteo, Osteosarcoma; P(d), deep parotid gland; P, parotid gland; pleomorph, Pleomorphic sarcoma; S, Submandibular gland; sal duct, Salivary duct carcinoma; spindle cell, Spindle cell sarcoma; squamous, Squamous cell carcinoma; undiff, Undifferentiated carcinoma.

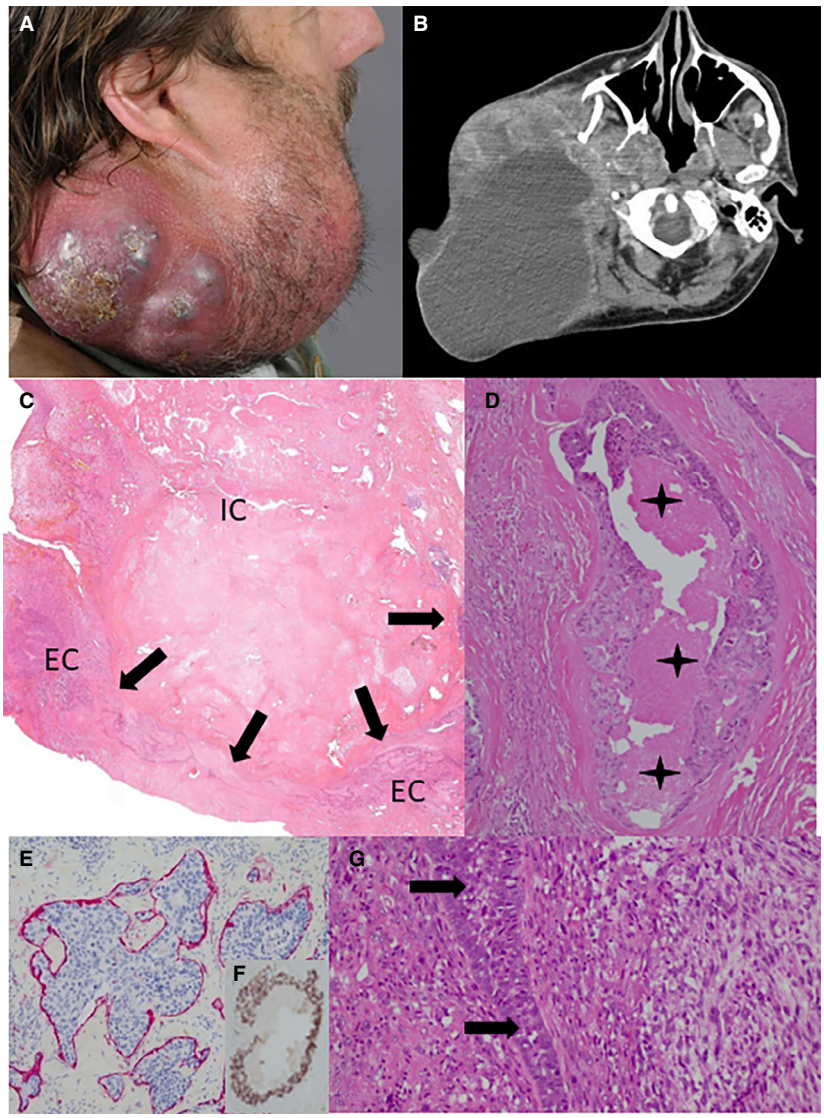

Figure 1. A 56-year-old man with a small parotid tumour for 15 years. (**A**) Now with rapid tumour growth and facial nerve palsy (case no. 1). **B**: Computer tomography with tumour of 150 mm diameter in the parotid area. **C**: Intensely sclerotic PA (arrowheads indicate capsule) with intracapsular (IC) carcinoma and extracapsular (EC) high-grade carcinosarcoma. **D**: Higher-magnification with intraductal carcinoma with comedo necrosis (stars), (**E**) embraced by CK14-positive myoepithelial cells (red), and (**F**, inset) with nuclear overexpression of p53 (brown). **G**: Combination of SDC (arrows) and pleomorphic sarcoma in extracapsular component.

Illustrated with different colours in Table 1, 15 cases could be assigned to two groups or pathways, respectively, with the following characteristics: the larger group (intraductal pathway; cases 1–9; 56.3%; Figure 1) displayed obligate histological and/or molecular evidence of PA and of intraductal carcinoma, combined with predominantly undifferentiated (n = 6) or salivary duct carcinoma (n = 5) epithelial component, and pleomorphic (n = 4), osteo- (n = 3) and chondrosarcoma (n = 4) morphology as a mesenchymal component. The smaller group (myoepithelial pathway; cases 11–16; 37.5%; Figure 2D) displayed histological and/or molecular proof of PA in 5/6 cases; however, there was a consistent absence of intraductal carcinoma, combined with almost obligatory combination of myoepithelial carcinoma (n = 5) and chondrosarcoma (n = 6). The two groups displayed no differences with respect to sex, age, type of gland, or pT-stage.

Most cases showed advanced stages, with pT3/4 in 11/16 and >6 mm depth of infiltration in 12/13 cases, with a medium diameter of 45 mm and a

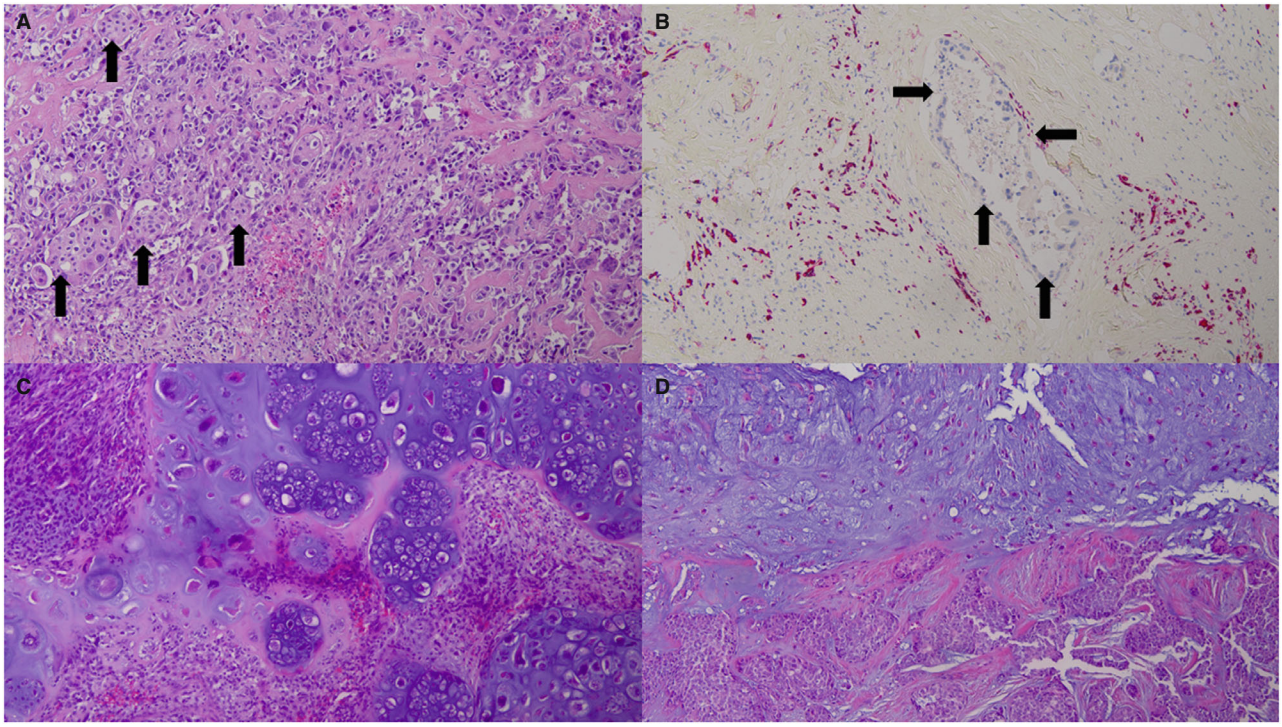

Figure 2. Examples of combined carcinomatous and sarcomatous tumour: **A**: Case no. 3 with dominant osteosarcoma and minor component of SDC (arrows). **B**: Case no. 7 with intraductal neoplasia (arrows) and myogenic sarcoma (desmin: Red). **C**: Case no. 8 with dominating chondrosarcoma and undifferentiated carcinoma. **D**: Case no. 13 with chondrosarcoma and myoepithelial carcinoma.

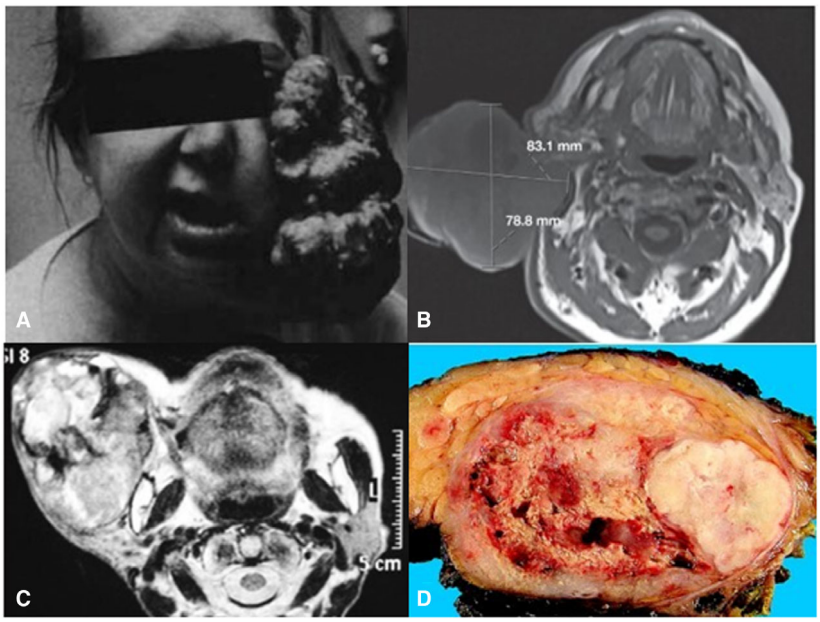

Figure 3. Historic reports of huge, exophytic parotid SCS (all permissions for reprint obtained). **A**: A 57-year-old female, death 2 months later, report from 1989.[5] **B**: A 77-year old-male with tumour of 83 mm diameter in magnetic resonance imaging, report from 2020.[13] **C**: A 76-year-old female with "rapidly expanding" tumour of 85 mm diameter in magnetic resonance imaging, report from 2015.[30] **D**: A 50-year-old male, macroscopic specimen with tumour of 65 mm diameter and hemorrhagic necrosis (left), report from 2013.[12]

diameter of 60–150 mm in the five largest tumours (Figure 1,3). Rapid growth and/or facial nerve palsy was frequently reported. Lymph node metastases were present and absent, respectively, in five cases each (unknown in six). Follow-up data were limited; two patients died of disease, one suffered from lung metastases, and one had no disease.

FINDINGS FROM THE LITERATURE

A literature search identified 78 publications describing 111 cases of SCS, the first in 1953. The majority (n = 69) represented single case reports, typically with limited follow-up, with nine publications describing 2 to 12 cases. The prevailing terminology until 1990 was (true) malignant mixed tumour,[1,2,6,7,9,10] while carcinosarcoma was regularly used thereafter.[3–5,8,11–14] Cases of SCS ex PA represented 3%–6% in large CEPA series.[2,20] The average age was 60.1 years (10–86), 57.3% were male, 70.3% in parotid, 18.9% in submandibular, and 11.8% in minor glands. While the average size was 39 mm,[1] a size >60 mm, often with rapid enlargement, was relatively often reported (Figure 3[4,5,8,9,12,13]). Four cases, all before 1990, occurred many years after radiotherapy due to PA.[10]

There were two malignant components (one carcinoma/sarcoma type each) in 66.3%, three in 19.8%, four in 8.1%, and five or more in 5.8%. The most frequent carcinoma types were adeno- (43.0%), salivary duct (29.1%), undifferentiated (26.7%), and squamous cell carcinoma (18.6%), while myoepithelial carcinoma represented 2.3%. Most frequent sarcoma types were chondro- (51.2%), osteo- (29.1%), spindle-cell (27.9%), pleomorphic (8.1%), and rhabdomyosarcoma (7.0%), with a heterologous component in 75.6%.[1–3,5,8,12,13] Concomitant PA was reported in 49.6%, alternative "*de novo*" development (dedicated absence of PA) was postulated in 24.3%, the presence or absence of PA was not commented on in 26.1%.[1,3,12] A description of intraductal/intracapsular malignant tumour was restricted to two cases.[8,12]

The estimation of prognosis was limited, as follow-up data were lacking in 53.2%. In the remainder, 7.2% were free and 9.9% alive with disease, 27.0% died of disease (mostly due to metastases to lung and/or liver[1–5,12]).

COMPARISON OF FINDINGS IN THE CURRENT CASE SERIES WITH THE LITERATURE

Table 2 summarizes important findings for SCS, comparing data of the case series and literature.[1–10,12–14,30] The most striking difference is the detection rate of PA, which is far higher in the series (93.8%) than in the literature (49.6%). Frequency of the dominating carcinoma and sarcoma components is similar, with the exception of myoepithelial carcinoma, which is frequent in our series (31.3%), but rare in the literature (2.3%). There are no relevant differences concerning sex, age, location, size (PA and SCS), and heterologous differentiation.

COMPARISON OF FINDINGS FROM OUR SCS SERIES WITH CEPA AND WITH CARCINOMA WITH SARCOMATOID DEDIFFERENTIATION

The findings in SCS and CEPA are very similar (Table 3), especially concerning age, sex, location, frequency/size of PA, and frequency of intraductal carcinoma. A striking difference is with pure intracapsular carcinoma/tumour, which is frequent in CEPA (36.5%[20]), but rare in SCS (6.3%). While being dominant in both entities, the intraductal carcinoma pathway is less frequent in SCS. While the overall size of SCS (mean 45 mm; present series) is only moderately

Table 2. Comparison of main findings in SCS between present case series and the literature

	Case series	Literature*
Proven PA (histological ± molecular)	93.8% (15/16)	49.6% (55/111)
Recurrent PA (in relation to cases with PA)	13.3% (2/15)	20.0% (11/55)
Most frequent carcinoma types	Undifferentiated (37.5%) and myoepithelial (31.3%) carcinoma	Adeno- (43.0%) and salivary duct carcinoma (29.1%)
Most frequent sarcoma types	Chondro- (75.0%) and pleomorphic sarcoma (37.5%)	Chondro- (51.2%) and osteo-sarcoma (29.1%)
Heterologous differentiation in sarcoma	81.3% (13/16)	75.6% (65/86)

*[1–7, 9–14, 30].

Table 3. Major similarities between SCS (present series) and CEPA (previous series reported by our group[19])

	SCS (Present series)	CEPA[19]
General frequency	very rare (16 cases)	rare (85 cases)
Development from PA	93.8% (15/16)	100% (85/85; by definition)
Frequency of recurrent PA	13.3% (2/15)	25.9% (22/85)
Size	Frequently > 60 mm	Rarely > 60 mm
Intraductal carcinoma (in cases with PA)	75.0% (9/12)	75.9% (60/79)
Pure intracapsular tumour stage	6.3% (1/16)	36.5% (31/85)
Intraductal carcinoma pathway	56.3% (9/16)	76.5% (65/85)
Myoepithelial carcinoma pathway	37.5% (6/16)	23.5% (20/85)

Table 4. Major differences between SCS (present series) and salivary carcinoma with sarcomatoid dedifferentiation (literature)

	SCS (Present series)	Salivary carcinoma with sarcomatoid dedifferentiation[15,16]
General frequency	Very rare	Rare
Size	Majority >40 mm	Mostly <30 mm
Development from PA	93.8% (15/16)	None (by definition)
Predominant sarcomatoid differentiation	Chondro- and pleomorphic sarcoma	Spindle cell and pleomorphic sarcoma
Heterologous differentiation	81.3% (13/16)	Very rare

larger than that of CEPA (39 mm[24]), huge tumours measuring >60 mm are more frequent in SCS (Figures 1, 3[13]).

Comparing SCS with salivary carcinoma with sarcomatoid dedifferentiation (Table 4), there are three major differences: While an association with PA is (almost) obligatory in SCS, it is absent in sarcomatoid carcinoma. Heterologous differentiation of the sarcomatous component is frequent in SCS (81.3% in the current study, 75.6% in the literature), but very rare in sarcomatoid carcinoma.[13,17] Tumour size is larger in SCS than in sarcomatoid carcinoma.

Discussion

The pathogenesis and terminology of SCS is still disputed: On the one hand, the relationship of SCS to PA and, accordingly to CEPA, and on the other hand the relationship between carcinomatous and sarcomatous components have been a subject of controversy, and molecular studies were lacking so far. Our morphophenotypic and molecular study on the largest series of 16 cases, combined with a thorough literature search, aimed to address these aspects.

In 111 cases identified in our literature search, PA had been described in 49.6%, while the alternative hypothesis of *de novo* development was advocated in 24.3%. However, in many cases PA might have been missed, especially due to limited sampling and destruction of PA by large SCS.[1–3,5 8,12,13] The presence of a well-circumscribed, highly sclerosed nodule within a background of salivary carcinoma as being indicative of preexisting PA has been widely accepted.[19] Moreover, a series of gene fusions, including the partners *PLAG1* and *HMGA2* have been established as specific genetic characteristics of PA.[13,27,28]

Applying a combined histogenetic approach with extensive sampling (average 11.6 blocks/case), appropriate immunohistochemistry, and additional molecular studies, we identified a preexisting PA in 15 of 16 cases (93.8%), which is far beyond the detection rate of 49.6% in the literature.[1,3] This finding strongly

indicates that SCS develops almost uniformly from PA and only rarely, if at all, *de novo*.[1]

Moreover, the arguments presented herein strongly favour a complex multistep tumorigenesis of SCS, which is reminiscent of that in CEPA.[19–23,25,26] In about 2/3 of cases, intraductal neoplasia within tubules of PA, exclusively comprising carcinoma, proved to be equivalent to the initial step of the pathogenesis in CEPA. While in the early stages of intracapsular tumour (intraductal + intracapsular-invasive) the carcinomatous components were quantitatively dominant, the sarcomatous components were clearly dominant in the extracapsular stage. Frequent concordant immunohistochemical overexpression/negativity of p53 and AR in intra−/extracapsular SCS, combined with frequent concordance in carcinomatous/sarcomatous components for p53, further support this pathogenetic analogy of SCS to CEPA.

The origin of the sarcomatous component in SCS from myoepithelial cells of PA[1,4,9] or from primary carcinomatous components[8,12,14] has been hypothesised. We argue that our data provide convincing proof that SCS principally starts as carcinoma (developing from PA, so far equivalent to CEPA), with subsequent acquisition of secondary, finally dominating, and frequently heterologous sarcomatous components.

As an additional, novel finding we identified two alternative pathways within this multistep tumorigenesis (Table 1): A more common 'intraductal pathway' displays obligate histological and/or molecular evidence of PA and of intraductal neoplasia, combined with dominant undifferentiated and/or salivary duct-type carcinoma as an epithelial, and with pleomorphic and/or osteo−/chondrosarcoma as a mesenchymal component. A second, less frequent 'myoepithelial pathway' displays histological and/or molecular evidence of PA in most cases; however, with constant absence of intraductal neoplasia and almost obligate combination of myoepithelial carcinoma and chondrosarcomatous differentiation (Figure 2D). In the early stages of SCS, these two pathways perfectly recapitulate the two analogous pathways in CEPA, in both entities with dominance of the intraductal pathway (Table 3).[19–23,26] These pathogenetic concepts in SCS are completely novel, as there are no comparable data in the literature, neither concerning progression of carcinoma-dominant to sarcoma-dominant (intra−/extracapsular) tumour, nor concerning these two pathways.

Data from our series and the literature[1,5,13] indicate that the size is larger in SCS than in conventional CEPA and, especially, that a huge size of >60 mm (Figures 1, 3) is characteristic of SCS. Taking into account the overall dominance of the sarcomatous components in the extracapsular stage, this difference is due to overgrowth of the sarcomatous component. While widely invasive CEPA is well known to be prognostically poor,[11,19–25] data indicate that SCS may be even more aggressive.[2,4,12] However, prognostic data are limited, both in the literature,[1–4,8,12] and in our series.

In our previous series of CEPA, a high percentage (36.5%) was restricted to pure intracapsular CEPA, associated with a favourable prognosis.[20] On the contrary, the frequency of early, intracapsular stage is much lower in SCS (6.3%; our series). This is due to the fact that sarcomatous components, being the prerequisite for a diagnosis of SCS, exhibit progression in the late, extracapsular stages.

Delimitation of SCS from salivary carcinoma (often SDC) with sarcomatoid dedifferentiation is not straightforward. Intracapsular components of SCS (and accordingly of CEPA[19,20,26]) with typically high-grade, cribriform intraductal carcinoma show a major histomorphological similarity to invasive SDC.[13,16,17] Therefore, the possibility of misinterpretation of SCS as sarcomatoid SDC, and vice versa, is obvious.[8,12,13,16,17]

The terminology of biphasic epithelial-mesenchymal malignancies has been continuously evolving after a long historical controversy. These tumours, albeit rare, may occur in any organ with varying frequency. Although the term carcinosarcoma has been historically used in almost all organs, it started to disappear in most classifications, based on the generally accepted view, that the sarcomatous component is verified to be derived from the epithelial component. Accordingly, the cumbersome term "sarcomatoid carcinoma with/without heterologous mesenchymal components" has emerged as a substitute in most organs.

However, the terminology of carcinosarcoma has survived in the WHO classification of uterine tumours and also in the current 4th[11] and upcoming 5th WHO classification of salivary tumours. At this point, there is no consensus as to which terminology would better reflect the tumour characteristics, and hence be recommended. Given that a substantial subfraction of SDC originate ex PA and nearly all SCS do as well, distinguishing sarcomatoid SDC ex PA from SCS is rather arbitrary. Hence, sarcomatoid SDC ex PA and SCS might best be looked at as the same entity. Similarly, the presence of a chondroblastic sarcomatous component, which is remarkably associated with

tumours with myoepithelial pathway, can be considered as myoepithelial carcinoma ex PA with chondrosarcomatous differentiation.

In summary, our findings strongly indicate that SCS pathogenetically develops virtually always (or in the vast majority) from PA, whereas the hypothesis of a *de novo* development does not exist. We demonstrate as a novel finding that SCS in early, intracapsular stages exhibits an identical multistep carcinogenesis to that well-established for CEPA, while clinical characteristics (large size, rapid progression) are related to sarcomatous overgrowth in extracapsular stages. We thereby identified two alternative histogenetic pathways (intraductal versus myoepithelial pathway), which in the early stages as well proved to be identical to the carcinogenesis of CEPA. While the current and upcoming WHO classification[11] identifies SCS as an independent entity, exhibiting minor overlap with CEPA, we suggest to regard SCS pathogenetically as a rare, special, and aggressive variant of CEPA with secondary sarcomatous overgrowth in the late stages.

Author Contributions

S. Ihrler, D. Stiefel, and A. Agaimy designed and performed the study research. S. Ihrler, D. Stiefel, R. Stoehr, and A. Agaimy analysed the data. S. Ihrler, P. Jurmeister, A. Sandison, N. Chaston, J. Laco, N. Zidar, L. Brcic, and A. Agaimy contributed cases. S. Ihrler, D. Stiefel, A. Sandison, and A. Agaimy wrote the article.

Funding Information

This work was supported by the grant "Wissenschaftliches Herausgeberkolloquium der MMW," Germany (Grant 2007).

Conflict of Interest

The authors disclose that they have no significant relationships with or financial interest in any commercial companies pertaining to this article.

Ethics

This study was approved by the Ethics Commission of the Ludwig-Maximilians-University, Munich, Germany (nr 20–558).

Compliance With Ethical Standards

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Acknowledgement

Open Access funding enabled and organized by Projekt DEAL.

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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