

Introduction: Carcinosarcoma (CS) is a tumor with components: epithelial (carcinomatous) and mesenchymal (sarcomatous), developing in the mechanism of epithelial-mesenchymal transition. It is known that the p53 defect is a frequent finding in a carcinosarcoma in different anatomical locations, additionally, in a subgroup of uterine CS MMR defect plays a role in the pathogenesis. The aim of this paper was to investigate the frequency of MMR and p53 aberrations in extrauterine CS.

Material and methods: Twenty eight extrauterine CS from the lung ($n = 8$), breast ($n = 6$), head and neck ($n = 5$), ovary ($n = 3$), urinary bladder ($n = 3$), adrenal gland ($n = 1$), skin ($n = 1$), and stomach ($n = 1$) were stained for hMLH1, PMS2, hMSH2, hMSH6 and p53. The pattern of expression was evaluated separately in carcinomatous and sarcomatous component.

Results: Immunostainings for hMLH1, PMS2, hMSH2 and hMSH6 were positive in all tumors. p53 defect was observed in 19 out of 28 samples (67.85%). In all cases except one (96.42%) there was a concordance between sarcomatoid and carcinomatous components.

Conclusions: MMR deficiency does not seem to play a role in the pathogenesis of extrauterine CS. p53 aberrant expression is frequent and almost always consistent in carcinomatous and sarcomatous component.

Key words: carcinosarcoma, p53, MMR.

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Immunohistochemical evaluation of mismatch repair proteins and p53 expression in extrauterine carcinosarcoma/sarcomatoid carcinoma

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Introduction

Carcinosarcomas (CS) are rare tumors which consist of two malignant components, namely epithelial (carcinomatous) and mesenchymal (sarcomatous). These neoplasms have been observed in various locations among which the most frequent is the uterus, where it is also known as mixed malignant Müllerian tumor (MMMT). Irrespective of the location these tumors show poor prognosis. The ambivalent morphologic presentation of CS raises questions about its origin. To date there have been proposed three main hypotheses aiming to explain the origin of this tumor: 1) the collision theory: carcinoma and sarcoma are two independent neoplasms which arise in the same location and at the same time accidentally together; 2) the combination theory: both components originate from a single stem cell which undergoes divergent differentiation; 3) the conversion theory: the mesenchymal component is derived from an epithelial one. Comparative genetic analyses performed on various CS revealed large overlaps of chromosomal aberrations in both tumor components [1, 2], which speaks in favor of the 2 and 3 hypothesis.

Epithelial-mesenchymal transition (EMT) is a process of losing the epithelial phenotype and acquiring increased migratory potential. Under physiological conditions EMT is involved in the formation of the body plan, regular differentiation of many tissues and tissue repair. Under pathological conditions EMT occurs in many carcinomas and is associated with increased aggressiveness, metastatic potential and chemoresistance. It is generally believed that occurrence of the sarcomatous component of CS is a result of complete EMT [3–6]. However, molecular pathways involved in the process of EMT in CS are not clearly understood and might differ in anatomical locations. For example in pulmonary CS EMT is probably initiated by an upregulation of c-Jun and a consecutive overexpression of Vimentin and Fascin [7], whereas in uterine CS the Wnt signaling pathway is deregulated and Akt/beta-catenin pathway activates Slug inhibiting E-cadherin expression [8].

Table 1. Summary of p53 and MMR alterations in current cohort of various carcinosarcoma

No.	Organ	Age	Sex	P53 IHC	MMR IHC	Epithelial component	Mesenchymal component
1	Skin	70	Male	N	N	SCC	Chondrosarcoma + osteosarcoma
2	Ovary	64	Female	AL	N	Serous carcinoma	Undifferentiated spindle cells
3	Ovary	67	Female	AD	N	Serous carcinoma	Undifferentiated spindle cells
4	Ovary	55	Female	AD	N	Serous carcinoma	Undifferentiated spindle cells
5	Adrenal gland	72	Male	N	N	Undifferentiated carcinoma	Undifferentiated spindle cells
6	Urinary bladder	67	Female	N	N	Urothelial carcinoma	Undifferentiated spindle cells
7	Urinary bladder	67	Female	N	N	Urothelial carcinoma	Spindle cells with myxoid stroma
8	Urinary bladder	61	Male	AL	N	Urothelial carcinoma	Undifferentiated spindle cells
9	Lung	75	Male	AL	N	SCC	Undifferentiated spindle cells
10	Lung	62	Male	N	N	Large cell neuroendocrine carcinoma	Angiosarcoma
11	Lung	65	Male	AL	N	SCC + adenocarcinoma	Chondroid differentiation
12	Lung	74	Male	AD	N	SCC	Fibrosarcoma
13	Lung	68	Male	N	N	Adenocarcinoma + large cell neuroendocrine carcinoma	Undifferentiated spindle cells + myofibroblastic
14	Lung	59	Male	AD	N	SCC	Undifferentiated spindle cells
15	Lung	69	Male	N	N	SCC	Undifferentiated spindle cells
16	Lung	71	Male	AD	N	Adenocarcinoma	Undifferentiated spindle cells
17	Esophagus	55	Male	N	N	SCC	Undifferentiated spindle cells
18	Esophagus	64	Male	AL	N	Anaplastic carcinoma	Undifferentiated spindle cells
19	Submandibular gland	91	Male	AD	N	SCC	Pleomorphic mesenchymal cells
20	Head and neck	66	Male	N	N	SCC	Chondroid differentiation
21	Maxila	36	Male	AD	N	SCC	Undifferentiated spindle cells
22	Breast	79	Female	AL	N	Invasive ductal carcinoma, NOS	Undifferentiated spindle cells
23	Breast	37	Female	AD	N	SCC	Osteosarcoma
24	Breast	69	Female	AL in C	N	Invasive ductal carcinoma, NOS	Undifferentiated spindle cells
25	Breast	60	Female	AD	N	Invasive ductal carcinoma, NOS	Undifferentiated spindle cells
26	Breast	69	Female	AD	N	Invasive ductal carcinoma, NOS	Undifferentiated spindle cells
27	Breast	64	Female	AD	N	Invasive ductal carcinoma, NOS	Spindle cells with myxoid stroma + large cell component
28	Stomach	60	Male	AD	N	Adenocarcinoma with neuroendocrine differentiation	Chondroid differentiation + leiomyosarcoma

IHC – immunohistochemistry, AD – abnormal diffuse, AL – abnormal loss, N – normal, C – carcinoma

Among all anatomical locations the molecular landscape and pathogenesis are best explored in uterine carcinosarcoma. It is well known that *TP53* mutations are essential for CS pathogenesis as the vast majority of CS show concordant overexpression of p53 in the carcinomatous and sarcomatous component [9–11]. Two recent studies have demonstrated an aberrant p53 staining pattern and defective mismatch repair protein (MMR) status in a subgroup of uterine carcinosarcomas [12, 13]. In one of them p53 aberrant IHC pattern and MMR status were mutually exclusive [13]. However, there are no data about MMR status in extrauterine CS. The aim of this study was the immunohistochemical analysis of p53 and MMR status in a cohort of extrauterine CS.

Material and methods

Cases of CS recorded at the University Clinical Centre in Gdansk from 2007 to 2015 were retrieved from the archive. Reviews of hematoxylin-eosin stained slides of each case were newly performed by two independent, board certified pathologists (PC, WB) in order to verify the diagnoses. Eventually, 28 formalin-fixed and paraffin embedded tissue blocks were analysed in our study, including tissues from lung ($n = 8$), breast ($n = 6$), head and neck ($n = 5$), ovary ($n = 3$), urinary bladder ($n = 3$), adrenal gland ($n = 1$), skin ($n = 1$), and stomach ($n = 1$) tumors.

The process of IHC and its evaluation has been described in a previous study [13]. Briefly, tumor samples were stained with antibodies against hMLH1 (Clone ES05),

PMS2 (Clone EP51), hMSH2 (Clone FE 11), hMSH6 (Clone EP49), and p53 (Clone DO-7), all ready to use (DAKO, Denmark). Staining was performed on a Dako autostainer according to the manufacturer's instructions. The slides of all specimens were microscopically evaluated by two experienced pathologists (PC, WB). In the case of MMR proteins, nuclear staining was considered positive, and lack of nuclear staining as negative (a sign of defective MMR). We considered a strong/diffuse (> 75% of tumour cell nuclei) and a completely negative staining as p53 defect indicative of its mutation, (missense and nonsense, respectively), whereas a patchy/scattered pattern was regarded as a marker of normal p53 function. All statistical analyses were performed using the Statistica 12 (Statsoft). Concordance between sarcomatoid and carcinomatous components were evaluated with Fisher's exact test and kappa test. Other categorical variables were compared by Fisher's exact test.

Results

Immunohistochemical staining patterns of each tumor, along with basic demographic data are shown in Table 1. 28 patients, 16 males and 12 females, were included in the study. Median age at diagnosis was 66.5 (range 36 to 91, average 64.85). Immunostainings for hMLH1, PMS2, hMSH2 and hMSH6 were positive in all tumors.

We detected p53 defects in 19 out of 28 samples (67,85%). In almost all cases (96,42%) there was a concordance between sarcomatoid and carcinomatous components. In the breast cancer cases the p53 defect was usually reflected by its hyperexpression, while in lung by its complete absence. The patient's characteristics are provided in Table 1.

Discussion

So far the disturbances in the MMR expression status and/or MSI status was known only in CS of the uterus [12–19], with the frequency between 3% [18] up to 41% [17]. Two large recent multicentre analyses showed 4% of deficient MMR expression [20, 21].

In two studies, p53 IHC aberration [18] and *TP53* mutation [19] coexisted while in our previous study we observed MLH1 IHC loss due to *hMLH1* promotor hypermethylation being mutually exclusive with an aberrant p53 staining pattern and *TP53* mutation [13].

MMR deficiency has not been described in extrauterine CS so far and our study has failed to showed it either. In a group of Lynch Syndrome patients with sarcomas there was one CS, however, it was located inside the uterus as well [22]. Our study included tumors of various sites. It has been postulated that CS/sarcomatoid carcinoma in a specific organ is similar either to ordinary carcinoma in this location rather than to CS in another anatomical location [6].

Only in sebaceous neoplasms of the skin there is a frequent loss of MMR, especially MSH2 [23], however, the sarcomatous transformation of these tumors is unknown. MMR-deficiency is infrequent in the lung, breast (around 2%) [24], head and neck (around 7%) [25], ovarian (around 4%) [26], urinary bladder (around 2%) [27], stomach

(around 6%) [28] and adrenocortical carcinoma (around 3%) [29] so the lack of MMR deficiency in our small groups of patients might reflect its frequency in carcinomas.

MMR deficiency is used as a predictive biomarker for the therapeutic efficiency of immunotherapy in solid neoplasms. Our cohort is small, but our results show that MMR probably cannot be used as a predictive marker in CS and other tests should be utilized. The frequent aberrations in the expression of p53 and the almost concordance between the epithelial and mesenchymal component are in line with previous reports [30–34].

Conclusions

We have observed a high frequency of aberrant p53 IHC expression in CS and their high concordance between the carcinomatous and sarcomatous components. MMR-deficiency was not observed in our group of 28 CS of variable anatomical locations so it does not seem to play any significant role in its pathogenesis, contrary to the uterine carcinosarcoma, at least what we can state based on our limited sample size.

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