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# DNA mismatch repair and *TP53* defects are early events in uterine carcinosarcoma tumorigenesis

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**Growing molecular evidence shows that uterine carcinosarcomas are clonal tumors. The carcinoma component has a dominant effect in the aggressive clinical behavior of these tumors. Defective DNA mismatch repair affects up to 30% of endometrial adenocarcinomas. The frequency and importance of defective DNA mismatch repair in the histiogenesis of uterine carcinosarcomas remains controversial. We studied the pattern and frequency of defective DNA mismatch repair and *TP53* alterations in the epithelial and mesenchymal components of 28 uterine carcinosarcomas. We found evidence of defective DNA mismatch repair in six cases (21%) with a concordance rate of 83% for carcinoma-sarcoma pairs ($\kappa = 0.887$, $P < 0.001$). Lack of immunostaining for the *MLH1* protein was demonstrated in both components in two of these tumors. *TP53* defects were evaluated by 17p deletion analysis and p53 immunostaining. Nineteen carcinoma (68%) and 18 sarcoma (64%) components had evidence of either *TP53* allelic loss or p53 overexpression. These defects proved clonal in 76% of cases ($\kappa = 0.602$, $P = 0.003$). Our results indicate that defective DNA mismatch repair and *TP53* defects are common early events in carcinosarcoma tumorigenesis. The high rate of concordance for these molecular defects between the carcinoma and sarcoma components adds to existing molecular evidence that carcinosarcomas are clonal malignancies.**
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Carcinosarcomas of the uterus account for 1–2% of all malignancies of the uterine corpus. The pathologic hallmark of these tumors is the presence of malignant epithelial and mesenchymal components. These mixed malignancies can affect any of the müllerian derivatives of the female reproductive tract. Despite their relatively low incidence, these aggressive tumors represent a significant clinical problem.[1] The overall survival at 5 years for all patients with cancer of the uterine corpus is 84% and only 31% for patients with a uterine carcinosarcoma.[2,3]

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There is clinical and molecular evidence suggesting that uterine carcinosarcomas are clonal malignancies.[4–10] Immunohistochemical studies of carcinosarcomas have suggested a common epithelial origin.[4] Studies in nude mice have demonstrated that carcinoma cells derived from a carcinosarcoma cell line can give rise to tumors that include both epithelial and mesenchymal components whereas sarcoma cells do not.[5] Furthermore, the epithelial and mesenchymal components frequently share patterns of X-inactivation, allelic loss, and *TP53* mutation.[6,7] Clinically, the carcinoma component is more frequently found in metastatic deposits,[4,8] leading most clinicians to approach this tumor as a poorly differentiated carcinoma rather than a sarcoma.

Defective DNA mismatch repair is a relatively common phenomenon in endometrial adenocarci-

nomas, affecting more than 25% of these tumors.[11] The microsatellite instability (MSI) tumor phenotype is a hallmark of defective DNA mismatch repair. Although MSI has been reported to be infrequent in uterine carcinosarcomas (~5%),[12,13] recent studies from our laboratory have suggested that MSI may be more common in uterine carcinosarcomas than previously described and that defective DNA mismatch repair may be a feature unique to the epithelial component of these tumors.[12–14]

We therefore sought to determine the DNA mismatch repair and *TP53* status in each component of uterine carcinosarcomas in an attempt to better understand the histiogenesis of these malignancies.

## Materials and methods

### Patient Population, Tissues and DNA Specimens

This study was approved by The Washington University Medical Center Human Studies Committee and all participants explicitly consented to participate in ongoing molecular studies. Tumor and blood samples were collected from these patients and demographic and clinical information was entered in a computerized database. Of the patients consented between 1993 and 2004, there were 28 with a pathologic diagnosis of uterine carcinosarcoma for whom archival tissue blocks and slides were available for review when this study was initiated. The mean age for these patients was 72±10 years (age±s.d.). Twenty-one cases (75%) were Caucasian, six were African-American (21%) and no race was available in one case. Histologic sub-type of the individual components and stage distribution are presented in Table 1.

Diagnostic slides were reviewed and the histopathologic classification of the cases confirmed by a single gynecologic pathologist (PCH). Areas of high neoplastic cellularity (≥70%) for carcinoma and sarcoma elements were identified and marked for tissue dissection and DNA extraction. Laser capture micro-dissection and/or needle dissection of mesenchymal and epithelial components of each tumor was performed from unstained 5 μm slides. Tissue was placed in 50 μl of LCM buffer (0.04% Proteinase K in 1 mM EDTA, 10 mM Tris HCl and 1% Tween 20 (pH 8.0)) and digested overnight at 37°C in a rotating incubator. The proteinase K was then inactivated at 95°C for 8 min. This DNA stock solution was then used for PCR amplification.

### MSI Analysis

MSI analysis of the individual epithelial and sarcomatous components was performed as previously described[15,16] using five National Cancer Institute consensus microsatellite markers.[17] Each component was evaluated individually and confirmatory MSI typings were performed up to three times as needed. Tumor components were designated as having high-level MSI (MSI-H) if novel PCR bands were present in at least two of the five consensus panel markers, low-level MSI (MSI-L) if a single marker demonstrated MSI or microsatellite stable if there was no evidence of MSI in any marker. Only MSI-H was considered diagnostic of defective DNA mismatch repair.

**Table 1** Histology and stage distribution of uterine carcinosarcoma cases

| | N *(%)* |
|---|---|
| *Carcinoma* | |
| Serous | 13 (46%) |
| Endometrioid | 11 (39%) |
| Serous and endometrioid | 3 (11%) |
| Undifferentiated | 1 (4%) |
| *Sarcoma* | |
| Leiomyosarcoma | 10 (36%) |
| Undifferentiated | 8 (29%) |
| Rhabdomyosarcoma | 4 (14%) |
| Chondrosarcoma | 2 (7%) |
| Mixed | 4 (14%) |
| *Stage* | |
| I | 11 (39%) |
| II | 2 (7%) |
| III | 7 (25%) |
| IV | 8 (29%) |

### Immunohistochemistry for MLH1, MSH2 and MSH6

Immunohistochemistry for MLH1, MSH2 and MSH6 was performed using 5 μm-thick paraffin sections mounted on charged slides and interpreted by an experienced pathologist (TBE). Specific antibodies were processed as previously described using the following concentrations: 1:200 for MLH1 (Clone G168–728; BD PharMingen, San Diego, CA, USA), 1:400 for MSH2 (Clone FE11; Zymed Laboratories, San Francisco, CA, USA) and 1:600 for MSH6 (Clone 44; BD Transduction Laboratories, San Diego, CA, USA).[18] Nuclear staining was read as positive and absence of nuclear staining was read as negative.

### Evaluation of TP53 Status: Loss of Heterozygosity for 17p and p53 Immunostaining

The status of *TP53* was evaluated indirectly by loss of heterozygosity (LOH) analysis and p53 immunohistochemistry. LOH analysis was performed using the consensus MSI marker D17S250, five previously described SNPs located within 200Kb of this marker (rs12602312, rs6503741, rs575809, rs4795339, rs1014263) analyzed by restriction digest (primers, amplification conditions as well as restriction endonucleases available upon request), and a newly designed dinucleotide repeat microsatellite marker 23CA (*forward primer*—TCTTGGCACATCTGAAAGCA, *reverse primer*—GTAACCGGCTGTGCTGTCTC, Tm: 60°C).

Immunostains for p53 were performed on 5 $\mu$m-thick sections using clone 1803 (BioGenex, San Ramon, CA, USA) at a dilution of 1:200 using pretreatment and staining with the Ventana BenchMark XT IHC/ISH Staining Module (Ventana Medical Systems Inc., Tuscon, AZ, USA) following manufacturers' recommended protocols. IHC results were interpreted for the individual components of each tumor by an experienced gynecologic pathologist (PCH). p53 IHC was considered positive when tumor cells demonstrated strong nuclear staining.

LOH for 17p or *TP53* overexpression (as determined by immunostaining) was considered evidence of a *TP53* defect.

### Statistical Analysis

Descriptive statistics were used to evaluate cohort, disease and molecular characteristics. Fisher's exact test and kappa test were used to evaluate associations and concordance. A *P*-value of $<0.05$ was considered significant.

## Results

### Defective DNA Mismatch Repair and TP53 Defects in Carcinoma and Sarcoma Pairs

Six out of 28 carcinoma-sarcoma pairs (21%) had evidence of defective DNA mismatch repair based on the MSI-H phenotype in at least one component (carcinoma or sarcoma). Twenty-one out of 28 pairs (75%) had suspected *TP53* defects as evidenced by either loss of heterozygosity for 17p (15/28 cases) or by p53 overexpression (15/26 cases) in either component. The majority of cases in our series (79%) were informative for clonality analysis. The MSI and *TP53* status for the entire cohort is presented in Table 2.

### Defective DNA Mismatch Repair in Carcinosarcomas

Five out of six tumors (83%) showed concordance for the MSI-H phenotype in both epithelial and mesenchymal components ($\kappa=0.887$, $P<0.001$). Lack of immunostaining for the *MLH1* protein was demonstrated in both components in two of these tumors (cases 1144 and 1689, Figure 1a). In four cases *MLH1*, *MSH2* and *MSH6* immunostains were normal, despite phenotypic evidence of defective DNA mismatch repair (MSI-H). There was no difference in the frequency of MSI-H between the carcinoma (5/28) and sarcoma (6/28) components.

### 17p LOH and p53 Overexpression

17p LOH was identified in at least one component of the tumor in 15 cases (54%). Specifically, LOH occurred in 12 of 27 (44%) carcinomas (it was not possible to determine 17p status in one carcinoma specimen—case 160) and 14 of 28 (50%) sarcomas. Most carcinoma-sarcoma pairs (73%) had identical LOH patterns and as such, were considered clonal for 17p deletion ($\kappa=0.705$, $P<0.001$).

p53 immunostaining was successful for 26 tumors. Overall, 15 cases (58%) demonstrated p53 overexpression. Fourteen carcinoma (54%) and 13 sarcoma components (50%) showed p53 overexpression. There was 80% concordance for p53 overexpression in the carcinoma-sarcoma pairs ($\kappa=0.769$, $P<0.001$).

Taken together, the findings for 17p LOH and IHC suggest *TP53* defects in at least 19 carcinomas (68%) and 18 sarcomas (64%). In most cases (75%), 17p LOH and *TP53* overexpression were consistent (Figure 1b). However, some cases demonstrated discordant p53 immunostaining despite clonal evidence of 17p LOH or vice versa (Figure 1c).

Overall, *TP53* defects proved clonal in 76% of cases ($\kappa=0.602$, $P=0.003$).

## Discussion

Previous molecular and clinical studies have suggested that uterine carcinosarcomas are clonal malignancies;[4–7] however, the roles defective DNA mismatch repair and *TP53* defects play in these

**Table 2** MSI phenotype and *TP53* defects in carcinosarcomas

| *Case* | *Carcinoma* | | *Sarcoma* | |
|---|---|---|---|---|
| | *MSI-H* | *TP53 defect* | *MSI-H* | *TP53 defect* |
| 165, 1161, 1208, 1239, 1338, 1467, 1469, 1491, 1595, 1596, 1634, 1728, 1765, 1860 | − | + | − | + |
| 1689 | + | − | + | − |
| 1643 | + | + | + | + |
| 160, 1210, 1354, 1461, 1630, 1850 | − | − | − | − |
| 1203, 1413 | + | + | + | − |
| 1144 | + | − | + | + |
| 1230 | − | + | + | + |
| 1828 | − | + | − | − |
| 1848 | − | − | − | + |

+: Indicate MSI-high phenotype or *TP53* defect by either 17p deletion analysis or immunohistochemical overexpression.

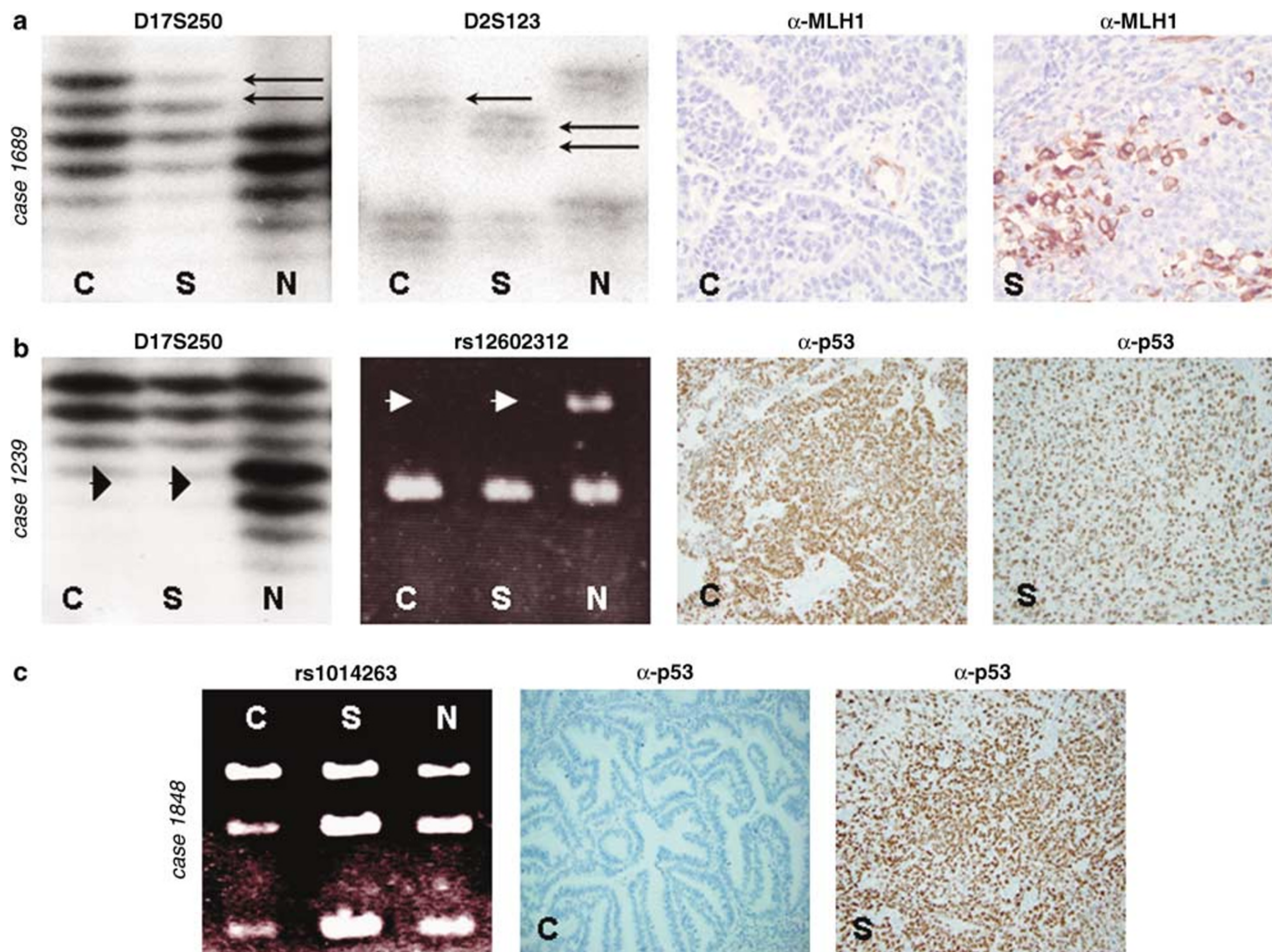


**Figure 1** Representative examples of molecular and immunohistochemical findings for carcinosarcomas. (**a**) Concordant MSI and MLH1 IHC in case 1689. Left two panels show abnormal PCR products characteristic of MSI (arrows) for D17S250 and D2S123 markers in both the epithelial and mesenchymal components. Lack of immunodectable MLH1 protein in the serous carcinoma and chondrosarcoma components of this tumor is illustrated in the right two panels (positive staining in endothelial cells in the sarcomatous element serves as internal positive control). (**b**) Concordant LOH at D17S250 and rs12602312 (Mae II RFLP) in the carcinomatous and sarcomatous elements of case 1239. Arrowheads indicate alleles lost in the cancer (left two panels). p53 overexpression in the serous carcinoma and mixed (rhabdomyosarcoma and undifferentiated) sarcoma from case 1239 (right two panels). (**c**) Retention of heterozygosity in both the carcinomatous and sarcomatous elements of case 1848 (left panel). Allelic retention of 17p shown by Cac8I restriction digestion of a 17p SNP (rs1014263). IHC demonstrates p53 overexpression in the leiomyosarcoma but not in the endometrioid carcinoma component (right two panels). C: carcinoma; S: sarcoma; α-MLH1: MLH1 antibody; α-p53: p53 antibody.

tumors have not been clearly defined. We observed a high overall rate of MSI (21%), 17p deletion (54%), and p53 overexpression (58%) among 28 carcinosarcomas investigated. In our study, both DNA mismatch repair and/or *TP53* defects were common to both components of carcinosarcomas. Concordance for the MSI-H phenotype was seen in 5/6 tumors (83%), and 76% of cases proved clonal for *TP53* defects as defined by 17p LOH or *TP53* overexpression. The high degree of concordance of *TP53* and/or DNA mismatch repair defects seen in this study provides additional evidence for a clonal origin in these malignancies.

The high frequency with which *TP53* and/or DNA mismatch repair defects are shared by the carcinomatous and sarcomatous elements suggest that these lesions occur early in the tumorigenesis (Table 2). Figure 2 is a schematic representation of where and when *TP53* and/or DNA mismatch repair defects occur in the genesis of carcinosarcomas based on our analysis of 28 tumors. In most cases, the progenitor cell that gives rise to both the sarcoma and carcinoma components acquires either a *TP53* defect (14/28 cases; 50%), loses DNA mismatch repair (case 1689; 4%) or acquires both defects (case 1643; 4%). When the initial event occurs in the progenitor cell these defects are shared by the epithelial and mesenchymal tumoral components. Alternatively, the progenitor cell may be affected only by defective DNA mismatch repair (defect present in both descending lineages) and only one compartment subsequently acquires a *TP53* alteration (cases 1144, 1203, 1413; 11%). *TP53* alteration in the progenitor followed by loss of mismatch

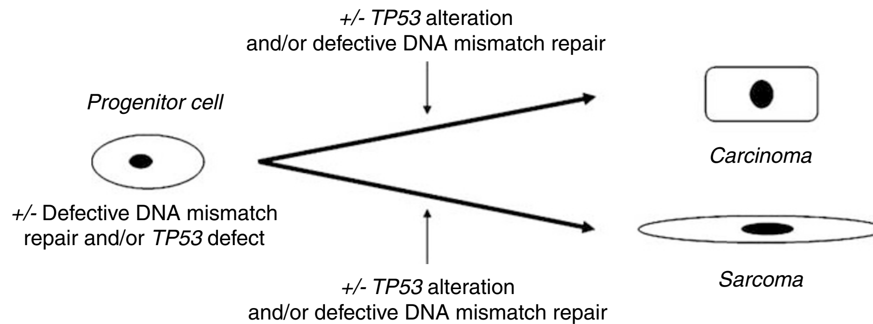


**Figure 2** Proposed model for histiogenesis of uterine carcinosarcomas. A normal progenitor cell may acquire a number of genetic mutations including defects in *TP53* and DNA mismatch repair. Additional defects are acquired as the tumor differentiates into the carcinoma and sarcoma components. Shared molecular defects (present in the progenitor cell) will be present in both components. Molecular defects acquired later in histiogenesis will be discordant between the two components.

repair in one compartment also occurs (case 1230; 4%). In some instances, a single cell lineage was affected by either one of these molecular alterations (in case 1828 and in case 1848; 7%) or no molecular lesion could be identified (6/28 cases; 21%). In the absence of definitive discordance the case for clonality could still be explained by other molecular mechanisms not explored in our study. Overall, this model supports clonality in at least 71% of our cohort based on identification of clonal molecular abnormalities.

We found a higher proportion of defective DNA mismatch repair in carcinosarcomas than was previously reported (21.4% *vs* 5%).[10] However, only two of six tumors with MSI-H had defects in *MLH1*, *MSH2*, or *MSH6* expression based on IHC. This may reflect DNA mismatch repair deficiency attributable to another member of this pathway such as *MLH3* or *PMS2* or missense changes in one of the proteins that abrogate repair. Alternatively, a novel DNA mismatch repair protein may be involved in carcinosarcoma tumorigenesis. Although unlikely, it is also possible that technical artifacts were responsible for false positive MSI results.

We also found a relatively high rate of p53 overexpression (58%) in this cohort of carcinosarcomas which is consistent with the range of 30–60% reported previously for these tumors.[19,20] p53 overexpression has been associated with biological aggressiveness in endometrioid adenocarcinomas as well as in papillary serous carcinomas of the endometrium.[21,22] As uterine carcinosarcomas are clinically aggressive malignancies and approximately 54% of the patients in our cohort had metastatic disease at presentation, it was not surprising to find a high rate of p53 overexpression in these tumors. *TP53* overexpression is known to be associated with carcinomas of the serous type. Although 16/28 tumors had serous carcinoma within the epithelial component, there was no association between *TP53* defects and the presence of serous carcinoma ($P = 0.114$).

Overall, our data suggest that DNA mismatch repair and *TP53* defects are early events in carcinosarcoma tumorigenesis. Furthermore, the high rate of concordance for these molecular defects among the carcinoma and sarcoma components adds to existing molecular evidence that carcinosarcomas are clonal tumors. Further investigation is needed to determine whether there is an association between DNA mismatch repair and/or *TP53* defects and clinical outcomes.

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