Advances in Brief

Prognosis Is Correlated with p53 Mutation Type for Soft Tissue Sarcoma Patients

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Abstract

We investigated the prognostic value of p53 mutation type for 145 soft tissue sarcoma patients. In a PCR-SSCP-sequencing analysis, 15 mutations were identified: 10 nonframeshift (non-fs) and 5 frameshift (fs) mutations. Patients possessing non-fs mutations had a significantly poorer prognosis than patients without p53 mutations (P = 0.014), according to Cox’s multivariate analysis. In contrast, the survival of five patients with fs mutations was not affected by their mutation type. Furthermore, occurrence of lymph node metastases and recurrences was correlated with the mutation type; i.e., 4 of 10 and 5 of 10 patients with non-fs mutations showed lymph node metastases and recurrences, respectively, whereas none of the patients with fs mutations showed lymph node metastases and only one a recurrence. Therefore, for the evaluation of prognosis, we suggest applying not the p53 mutational status in general, but the specific type of mutation.

Introduction

It is generally accepted that p53 mutations in human malignant tumors are often related to a poorer prognosis (1–3). This relationship raises two questions: (a) are there differences in the distribution pattern of mutations and do these influence their efficacy? and (b) is there a difference in the efficacy of individual mutation types?

The first question has already been partially answered: about 90% of p53 mutations within malignant tumors are localized within the highly conserved core domain, which roughly corresponds to exons 5–8 of the p53 protein (4, 5). The distribution of individual mutation types differs significantly between exons 5–8 (79% missense mutations) and exons 2–4 and 9–11 (predominantly non-missense mutations; Refs. 6, 7). The functional efficacy of point mutations varies depending on their location. Tumors with point mutations within the highly conserved domains of the p53 gene are inherently more aggressive than tumors with point mutations outside these domains (3, 8).

The second question concerning the efficacy of mutations in relation to the mutation type has, in contrast, hardly been examined at all. One would expect differences based on whether the p53 protein remains functionally active after a mutation or not [i.e., whether (a) a mutation produces an unchanged reading frame (non-fs mutation) and thus an active p53 protein, which can act dominant negative through binding and inactivating wild-type p53 (9, 10), or (b) through a shift in the reading frame, either a shortened p53 protein is produced or it disappears totally (loss of function)].

To our knowledge, differences in tumor behavior and resulting differences in prognosis depending on the type of p53 mutation have not yet been described.

Materials and Methods

Altogether, we examined 190 tumor samples from 145 STS patients (Institute of Pathology and Surgical Clinic, University of Halle; partially described in previous studies; Refs. 13 and 14) consisting of 30 malignant fibrous histiocytomas, 28 malignant neural tumors, 24 liposarcomas, 22 fibrosarcomas, 21 leiomyosarcomas, 6 rhabdomyosarcomas, 4 synovial sarcomas, and 10 other STS. For examination, we included tumors from different locations (extremities, 54%, intrathoracic/mediastinal/intraabdominal/intraperitoneal, 27%, trunk wall, 11%, head/neck, 8%). Insofar as it was possible, surgical therapy for all patients was compartment resection or resection with a safety margin. Histopathologic grading of the primary tumors showed 12% grade 1, 44% grade 2, and 44% grade 3 primary tumors (n = 145). In addition to the primary tumor, lymph node metastases (N2) were diagnosed for 16% of patients (patient selection through referral to a medical center), and distant metastases (M2) for 7% of patients. Eighty-three patients were treated with radiotherapy, 29 patients received a systemic chemotherapy, and 22 patients received both therapies. Out of 145 STS patients, 63% died of the tumor after an average of 23 months (range, 1–99 months), whereas 37% of the patients are alive after an average observation period (after primary tumor operation) of 54 months (range, 1–168 months).

The tumor samples were examined for mutations in the p53 gene by nonradioactive PCR-SSCP sequencing. Briefly, exons 4–9 were amplified in separate PCR reactions from DNA isolated from formalin-fixed paraffin-embedded or frozen tumor samples as described previously (13, 14). In a prescreen for mutations, PCR products were analyzed in 6 or 10% acrylamide ready-made gels (Novex, San Diego, CA) for abnormal single-strand DNA shifts, and striking cases were cycle sequenced with the help of the 5′-biotinylated PCR primers and detected by chemiluminescence (CPD-Star, Tropix, Bedford, MA).

The Cox regression model, which was used to estimate the effect of p53 mutations on survival, was adjusted to the prognostic effects of grading, location, and surgical resection radicality of primary tumors. The analysis was carried out using software from SPSS Inc.

Results and Discussion

In 15 of 145 STS patients we identified p53 mutations (Table 1). Ten were non-fs mutations (7 missense, 2 deletions, and 1 duplication in frame) and five were fs mutations (4 deletions and 1 insertion). Between the two groups of STS patients with p53 mutations (fs and non-fs), the distribution of mutations across the various exons was different. In our study, we mainly concentrated our attention on the effector mutant p53, i.e., on the individual mutations and their effect on the patient's survival. We chose STS as a model to investigate the efficacy of individual mutation types. To date, only a general correlation between an occurrence of p53 mutations and a poorer prognosis could be verified in STS (11, 12). However, STS provides a particularly good model for the examination of the tumor biological efficacy of different mutation types, due to the high percentage (>30%) of fs mutations (13, 14). In contrast, in epithelial malignant tumors the percentage is usually <10% (5).

In the course of our examination of STS, we were able to prove prognostic differences in dependence of mutation type. Only patients with non-fs mutations have a considerably poorer prognosis than patients without p53 mutations. fs mutations within STS, on the other hand, evidently do not influence the prognosis of the patients affected.
Table 1 Survey on p53 mutational status and clinical data for STS patients

<table>
<thead>
<tr>
<th>Mutation*</th>
<th>STS sample</th>
<th>Exon</th>
<th>Codon</th>
<th>Alteration bp alteration</th>
<th>Amino acid alteration</th>
<th>Entity</th>
<th>Sex</th>
<th>Age</th>
<th>N</th>
<th>R</th>
<th>M</th>
<th>RT/CT</th>
<th>Timea</th>
<th>Survival</th>
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<tr>
<td>Non-fs</td>
<td>M42</td>
<td>4</td>
<td>106–111 del</td>
<td>−(15)</td>
<td>5-aa del</td>
<td>LMS</td>
<td>F</td>
<td>67</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>24</td>
<td>d</td>
</tr>
<tr>
<td>P22/G25–92</td>
<td>5</td>
<td>177–182 del</td>
<td>−(18)</td>
<td>6-aa del</td>
<td>MFP</td>
<td>F</td>
<td>73</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>20</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>G251–92</td>
<td>5</td>
<td>nd</td>
<td>dp</td>
<td>+(42)</td>
<td>14-aa-dp</td>
<td>LS</td>
<td>M</td>
<td>31</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>40</td>
<td>a</td>
</tr>
<tr>
<td>M44/45</td>
<td>5</td>
<td>158</td>
<td>ts</td>
<td>CGC→CAC</td>
<td>Arg→His</td>
<td>LMS</td>
<td>F</td>
<td>61</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>6</td>
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</tr>
<tr>
<td>L17/18</td>
<td>7</td>
<td>237</td>
<td>tv</td>
<td>ATG→ATT</td>
<td>Met→Ile</td>
<td>LS</td>
<td>M</td>
<td>75</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>26</td>
<td>d</td>
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<tr>
<td>M24/25</td>
<td>7</td>
<td>245</td>
<td>ts</td>
<td>GGC→AGG</td>
<td>Gly→Ser</td>
<td>LMS</td>
<td>M</td>
<td>68</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>11</td>
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</tr>
<tr>
<td>M28</td>
<td>7</td>
<td>245</td>
<td>ts</td>
<td>GGC→AGG</td>
<td>Gly→Ser</td>
<td>Rhab</td>
<td>M</td>
<td>22</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>16</td>
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<tr>
<td>M19/20/21</td>
<td>7</td>
<td>245</td>
<td>ts</td>
<td>GGC→AGG</td>
<td>Gly→Ser</td>
<td>Rhab</td>
<td>M</td>
<td>58</td>
<td>−</td>
<td>+</td>
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<td>+</td>
<td>13</td>
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<tr>
<td>L11/12</td>
<td>7</td>
<td>248</td>
<td>ts</td>
<td>CGG→CAG</td>
<td>Arg→Gln</td>
<td>LS</td>
<td>M</td>
<td>65</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>12</td>
<td>d</td>
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<tr>
<td>P10/50</td>
<td>8</td>
<td>280</td>
<td>ts</td>
<td>AGA→GGA</td>
<td>Arg→Gly</td>
<td>MFP</td>
<td>M</td>
<td>55</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>7</td>
<td>d</td>
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<tr>
<td>L56</td>
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<td>54</td>
<td>del</td>
<td>−(9)</td>
<td>13-aa-dp</td>
<td>LS</td>
<td>M</td>
<td>49</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>46</td>
<td>a</td>
</tr>
<tr>
<td>GS4–92</td>
<td>4</td>
<td>69</td>
<td>del</td>
<td>−(1)</td>
<td>fs</td>
<td>MFP</td>
<td>F</td>
<td>75</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>39</td>
<td>a</td>
</tr>
<tr>
<td>P48</td>
<td>5</td>
<td>176/177 del</td>
<td>−(1)</td>
<td>fs</td>
<td>MFP</td>
<td>F</td>
<td>55</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>148</td>
<td>a</td>
<td></td>
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<tr>
<td>G14–93</td>
<td>6</td>
<td>204–212 del</td>
<td>−(25)</td>
<td>fs</td>
<td>MFP</td>
<td>M</td>
<td>85</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<td>a</td>
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</tr>
<tr>
<td>P22/G–93</td>
<td>6</td>
<td>215</td>
<td>ins</td>
<td>+(1)</td>
<td>fs</td>
<td>LMS</td>
<td>M</td>
<td>61</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>35</td>
<td>a</td>
</tr>
</tbody>
</table>
| *Mutation, type of identified mutation; N, lymph node status at primary tumor resection; R, number of recurrences; M, metastasis developed during follow up; RT/CT, radiotherapy/chemotherapy; ND, not determined; del, deletion; dp, duplication; ts, transition; tv, transversion; ins, insertion; LMS, leiomyosarcoma; MFP, malignant fibrous histiocytoma; LS, liposarcoma; Rhab, rhabdomyosarcoma; m, male; f, female; d, dead; a, alive.

a Time in months (follow-up after primary tumor resection).

b Patient did not die because of systemic metastases but because of multiple recurrences (head).

c All five fs mutations result in a stop signal (at least in exon 7); the functionally important oligomerisation domain and three COOH-terminal core localization signals get lost.

d Patient had one local recurrence six months after first operation (primary tumor) because of an initially incomplete tumor resection (R1).

non-fs mutations) differences could be established concerning lymph node metastases, recurrences, and survival. Among the patients with fs mutations, none showed a lymph node invasion at the time of the primary tumor operation. Strikingly, 4 of 10 patients (40%) with non-fs mutations already showed lymph node metastases at the time of the primary tumor operation, although in general these occur very rarely at a rate of only about 5% in sarcomas (15). For three patients, the lymph node metastases could be examined, and the non-fs mutation found within the primary tumor was also determined within the lymph node metastasis. Five of 10 patients with non-fs mutations developed recurrences after Rx resection. In contrast, only one patient with fs mutation showed a recurrence; this was proved to be due to an initially incomplete primary tumor resection.

Of the 130 patients without p53 mutations, 82 died after an average of 24 months (range, 1–99 months). Of the 10 patients with non-fs mutations, 9 died of the tumor after an average period of 15 months (range, 6–26 months) after primary tumor operation (Table 1). In contrast, five patients with fs mutations are alive after an average observation period of 60 months (range, 31–148 months) and have been free of metastases thus far.

We used a multivariate Cox model, including grading, site, and surgical radicality in the removal of the primary tumor, and found a statistically significant differing prognosis for patients with non-fs mutations in contrast to patients with p53 mutations (P = 0.014; relative hazard, 2.42). Thus, the non-fs mutation type proves to be an independent factor for a poor prognosis for STS patients (Fig. 1). In spite of the small number of patients (n = 5) with fs mutations, the trend suggests that this mutation type does not negatively influence the prognosis. For this reason, for the evaluation of prognosis we suggest taking into account not the p53 mutation in general, but the specific type of mutation.

Of clinical interest is the relationship between postoperative treatment, such as radiotherapy in the tumor bed and/or systemic chemotherapy, and prognosis.

The average number of patients treated with radiotherapy was comparable to those without mutations (74 of 130) and the two groups with p53 mutations (6 of 10 with non-fs and 3 of 5 with fs). In the group of patients without mutations, 47 of 74 patients (64%) with radiotherapy and 35 of 56 patients (63%) without radiotherapy died. However, in the group with non-fs mutations, almost all patients died and all patients with fs-mutations are still alive independent of receiving radiotherapy. Furthermore, the necessity for applying chemotherapy was given for 25 of 130 patients (19%) without p53 mutations and for 4 of 10 patients with non-fs mutations, but for none with fs mutation (no patients with metastasis). In the group without mutations, 21 of 25 of the patients treated with chemotherapy (61 of 105 without chemotherapy) died. All four patients with non-fs mutations treated with chemotherapy died.

Recently it was shown that p53 mutations, located in the Zn-binding region, are associated with de novo resistance to doxorubicin and early relapse in breast cancer patients (16).

In conclusion, knowledge of a patient’s p53 mutational status, with respect to both its location and the type of mutation, may give the opportunity to predict the clinical course of the disease and might have impact for the clinical treatment of patients.

Acknowledgments

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References


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