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Minireview

Soft Tissue Sarcomas and p53 Mutations

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Introduction

Soft tissue sarcomas (STS) are malignant tumors of mesenchymal origin. Despite the fact that soft tissues and bone comprise almost two-thirds the mass of the human body, sarcomas are relatively rare, with an incidence of about 1% (1). They represent a large, histologically diverse group of tumor entities, with a biological behavior difficult to predict, and they prove rather resistant to irradiation and/or chemotherapy (1). Clinical–pathological characteristics are well recognized as prognostic factors; these include grading (2,3), tumor entity (4–6), tumor localization (5,7), and tumor size (7–9). However, a correlation between tumor characteristics and behavior of STS is not always possible; large tumors can be associated with several relapses and are difficult to operate but exhibit no metastases; small tumors without relapses may be easy to operate but they metastasize quickly. Therefore, a reliable prognostic evaluation for individual STS patients and their individualized treatment is still awaited.

Tumorigenesis has been understood as a multistep process for 40 years (10). In 1989, molecular alterations were unraveled as the basis for carcinogenesis (11,12). These alterations occur in a number of genes involved in the control of cell proliferation, cell differentiation, and cell death (13). Two groups of genes in particular, tumor suppressor genes (TSG) and oncogenes, are affected. Oncogenes encode for proteins that are mostly elements of the signal transduction chain of growth factors, whereas TSG are translated into proteins with growth-inhibitory and differentiation-inducing functions (11,14). Mutations in both gene groups add up to a number of critical events in a malignant tumor (13,15).

Besides the retinoblastoma gene (Rb), one of the most prominent tumor suppressor genes is *p53*. The p53 protein controls central physiological processes such as transcription, cell cycle arrest, DNA repair, chromosomal segregation, genomic stability (the so-called guardian of the genome), cell differentiation, and apoptosis (16–20). Altogether, an incidence of p53 mutations in about 50% of malignant neoplasias strongly suggests diagnostic, prognostic, and therapeutic implications for malignancies (21,22).

However, knowledge about the prognostic relevance of p53 mutations in STS is not comprehensive (23). This review will not cover p53 germ-line mutations (recently reviewed in ref. 24), but it will focus on somatic p53 mutations, their distribution in different STS entities, and their prognostic relevance for individual patients with STS.

Frequency in Different STS Entities

Differences in the frequency of p53 mutations could lend weight to a role of p53 alterations in tumorigenesis in different STS entities. Altogether, a mutational rate of about 16% in STS is reliable, based on data of 142 p53 mutations from a data bank and recent publications (25–28). The diverse entities of sarcomas can be divided into two groups: (*1*) STS with a p53 mutational frequency of <5%, and (*2*) those STS with a mutational rate of >10%. The former group includes synovial sarcoma, fibrosarcoma and neuroblastoma; the latter consists of the other compiled STS (Table 1).

The frequencies of p53 mutations in the latter group suggest a considerable importance of p53 mutions in its tumorigenesis. This is sup-

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Table 1. Frequency of p53 mutations in different STS reported through 1997

Entity	Patients	Tumors	Total	With p53 Mutation	Frequency in %
Synovial sarcoma	7	8	15	0	0
Neuroblastoma	77	53	130	5	3.9
Fibrosarcoma	29	12	41	2	4.9
MFH	80	143	223	26	11.7
MPNST	30	34	64	8	12.5
Liposarcoma	67	54	121	21	17.4
Mesothelioma	17	4	21	4	19.1
Leiomyosarcoma	46	75	121	23	19.0
Rhabdomyosarcoma	49	17	66	16	24.2
PNET (without Nb)		20	20	8	40.0
Angiosarcoma	6		6	3	50.0
Mixed-mesenchymal tumors		41	41	24	58.5
Undifferentiated sarcoma	1		1	1	
Total	409	461	870	142	16.3

MFH, malignant fibrous histiocytoma; MPNST, malignant peripheral nerve sheath tumor; PNET, primitive neuroectodermal tumor; Nb, neuroblastoma. Compilation is based on data of a p53 mutational bank (25) and on further results (26–28).

ported by the frequent occurrence of sarcomas (24%) in patients with p53 germ-line mutations (29,30).

Localization of the Mutation

Localization of p53 mutations is of interest because most of the tumor-related mutations in carcinomas are detected in the core domain (amino acids 120 to 292), which contains mutational hot spots (codons 175, 245, 248, 249, 273, 282) (31). Although this finding is reasoned partially by a concentration on this region in mutational analyses, this region involves the residues structurally and functionally engaged in p53–DNA interaction that is essential for its function as transcription factor (32,33). Furthermore, prognostic findings show that mutations within the highly conserved domains are inherently more aggressive than mutations outside these domains (34,35). A search for tumor-specific mutational hot spots could give insight into tumor origin and allow a more efficient diagnostic screening. Codon 249 is recognized as the mutational hot spot for hepatocellular carcinomas in combination with aflatoxin B (36–38). Radon-specific mutations in codon 249 have been suggested by some investigators (39,40), but these have not been confirmed by others (41,42). Furthermore, a direct correlation between binding of cigarette smoke carcinogen derivates (benzo-[a]pyrene diol epoxide) to *p53* gene (guanine of codons 157, 248 and 273) and p53 mutations provides a direct ethiological link between a defined chemical carcinogen and lung cancer (43). Results from a study on the therapeutic implications of mutational localization indicate that de novo doxorubicin resistance is related to p53 mutations (in the zinc-binding region) (44).

In STS, specific mutational regions are not obvious. However, as an increasing number of mutations for p53 has been reported the described mutational hot spots have also started to crystallize for STS (Fig. 1). Until now, about one-fifth (29/142; 20.4%) of the known p53 mutations in STS concern mutational hot spots linked to carcinomas and lymphomas (45).

In addition to investigation of the core domain for p53 mutations, attention has focused on adjacent N- and C-terminal regions. The transactivation domain (amino acids 1 to 42) is located N-terminally and mutations there abrogate transcriptional activation (46). Furthermore, a proline-rich region (amino acids 61 to 94) capable of interacting with SH3 domains (Src homology domain 3) is important for binding to the oncogene c-*Abl*, which is induced after DNA

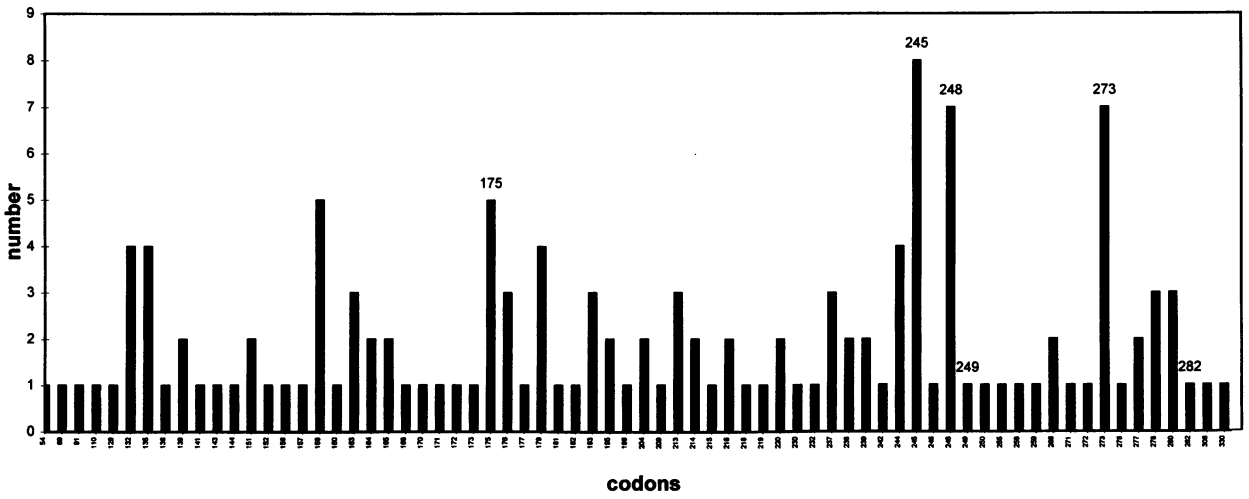

Fig. 1. Distribution frequency of p53 mutations in STS. All codons with p53 mutations are compiled. Six mutational hot spots described for carcinomas and lymphomas are numbered above the columns.

damage and involved in *p53* activation and cell-cycle arrest (19). Mutations in this region reduce the ability of the p53 protein to mediate apoptosis or cell-cycle arrest (47). The oligomerization domain and the nucleus localization signals are located C-terminally and mutations disturb protein assembly. In STS, only four mutations with a location in the N-terminal region (48,49) and two mutations for the C-terminal portion are described (48,50), pointing to an underestimation of mutational events and the necessity of further studies in these regions.

Kind of Mutation

The kind of mutation can give insight into the functional properties of the protein affected. For p53, which acts as a tetrameric protein, point mutations are characteristic (21,31,45,25), whereas in adenomatous polyposis gene (APC), which is a monomeric tumor suppressor protein, deletions and insertions are frequent (51). In p53, a single-point mutation can act dominant negatively affecting the DNA-binding and transcriptional transactivation function of the wild-type protein (52–54). Knowledge of the kind of mutation may also allow a correlation of mutation spectra to specific damaging agents, as was shown for ultraviolet (UV) radiation, with specific tandem CC to TT transitions (55), or for tobacco-associated cancers, which are associated with prevalent G:C to T:A transversions (45).

In STS, the majority of mutations are, as expected, point mutations (88%), including missense mutations (73%), nonsense mutations (8%), splice mutations (4%), and silent mutations (3%), followed by deletions (9%) and insertions or duplications (3%). Most of the point mutations are G:C (C:G) to A:T (T:A) transitions (64%), followed by G:C (C:G) to T:A (A:T) transversions (21%) and A:T (T:A) to G:C (C:G) transitions (17%). More than half of the former transitions (56%) occur at CpG sites, accounting for a quarter of all known *p53* mutations in STS (25). It is assumed that endogenous rather than exogenous mechanisms are responsible for mutations at CpG sites. CpGs are often methylated (5-methylcytosine) and sites for de-amination occur spontaneously or are induced by oxygen radicals or nitric oxides (56–58). Resulting mutations at these sites are less recognized and repaired (59), which supports the hypothesis of Cooper and Youssoufian (60) that mutations in CpG sites are a presumptive cause for human diseases.

Type of Mutation and Prognosis

Besides the correlation between localization and prognostic implications, it appears useful to correlate the type of p53 mutation with prognosis (49). In previous studies we demonstrated that STS patients with tumors possessing non-frameshift mutations showed a considerably poorer prognosis than patients without p53 mutations. In a multivariate Cox-Regression model for the former patients, an increased risk for tumor-caused death of 2.42 ($p = 0.014$) was calculated. In contrast, for patients with tumors bearing

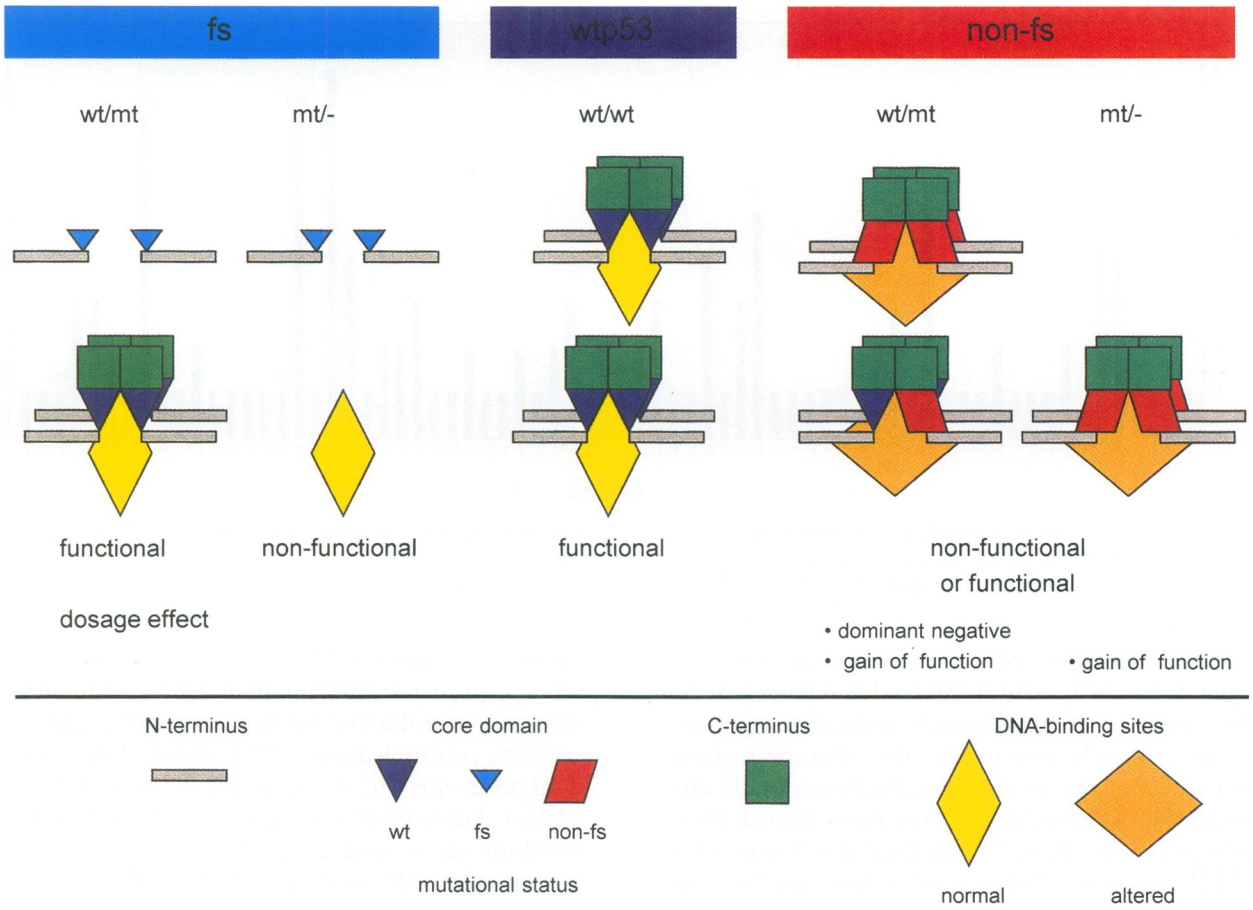

Fig. 2. Model investigating how mutational status affects p53 DNA-binding function. This model suggests that p53 mutation type can affect p53 protein assembling and therefore functional characteristics of DNA binding. Frameshift mutations (fs) result in a shortened or missing protein that is unable to oligomerize with the wild-type protein. As long as a wild-type protein is still present, it could function on a reduced dosage (left section). Presence of a non-frameshift mutation (non-fs) causes a mutant protein that can oligomerize. In addition to a functional loss because of the mutation other possibilities are described that are dependent on the mutational sites and events. The mutant protein can oligomerize with wild-type protein, thereby exerting a dominant negative effect at binding to the normal DNA binding sites or gain a new function at binding to other (altered) DNA binding sites. Alternatively, it can bind to another mutant p53, giving rise to a gain-of-function protein (right section). Furthermore, changed protein conformation could alter transactivation properties of other proteins.

frameshift mutations (and still a detectable wild-type allele), prognosis seemed to be unaffected (49). Furthermore, a relationship between mutation type and occurrence of relapses and lymph node metastases was obvious. About one-half of patients with tumors carrying non-frameshift mutations developed relapses and/or lymph node metastases. All except one patient with frameshift mutation tumors were not affected by further tumor occurrence. A comparably increased frequency of relapses at the presence of p53 mutations (unfortunately without interpretation of mutation type) is reported for head and neck squamous-cell carcinoma (61).

A possible explanation for this mutational behavior could be that, in the case of non-frameshift mutations, a mutated p53 protein may have a dominant negative effect on wild-type p53 (54), whereas in the case of frame-shift mutations, a truncated or missing mutated p53 protein might not affect the wild-type protein (Fig. 2).

Are p53 Mutations an Early or Late Event and Do They Contribute to Further Development of Cancer?

In a number of tumors, p53 mutations are often considered an early event because of their occur-

rence in early-stage tumors and precursor lesions, as in cancers of the lung, head and neck, breast, brain, and esophagus. However, in cancers of endometrium, cervix, ovary, liver, and bladder and in chondrosarcomas, p53 mutations have been detected in a rather advanced stage (45,62–64).

In STS, no precursor lesions are known, except for malignant peripheral nerve sheath tumors (MPNST) that can develop from neurofibromas (1). Therefore, in STS the distinction between early and late events seems inapplicable. However, p53 mutations were detected in both lower- and higher-grade STS (26,65,66).

We found a tendency of a somewhat earlier appearance of STS with p53 mutations (average age 59 years) versus the usually described maximum at 64 years, but differences in the mean age of occurrence depending on the STS entity are well known (1,67). Furthermore, in three informative cases we detected the same p53 mutation in the primary tumor and in the lymph node metastasis, which suggests a mutational event preceeding metastasis. Altogether, the early-aged occurrence of sarcomas with the presence of germ-line mutations points toward p53 mutations as an initial event in sarcomagenesis (24).

Importance of p53 Mutations in Clinical Practice

There is evidence from in vitro studies and from clinical approaches that p53 status is a crucial factor for successful treatment of malignancies. It has been shown that cells with mutated p53 or without p53 react less sensitively to radiation or chemotherapy (68–71) and cytotoxicity of adjuvant therapies depends considerably on p53-dependent apoptosis (72,73). Furthermore, it is interesting to note that tumors with no p53 mutations (testicular teratocarcinoma) or most often, with wild-type p53 (acute lymphoblastic leukemias) respond well to chemotherapeutic treatment. Tumors with high mutational rates for p53 (melanoma, lung cancer, colorectal tumors, and bladder and prostate cancer) often respond poorly to radiation and chemotherapy (19). This is not always the case, however, and it is not merely a simple relationship between p53 status and therapy response, because there are also reports of tumors with p53 defects that react (more) sensitively to radiation and chemotherapy (74–77). Additionally, in vitro studies have shown that a loss of p53 function may influence apoptosis ability, depending on the type of tumor cells and radiation dosage (78–80). In recent studies, 60 cell lines (40 with p53 mutations) were investigated for the effect of radiation (6.3 or 12 Gy) and that of 123 types of chemotherapy on cell growth inhibition and the induction of target genes. The majority of the p53-mutated cell lines showed a decrease in growth inhibition and target gene activation in comparison to the p53 wild-type cell lines, suggesting a p53-mediated influence on treatment efficacy. Noticeably, only chemotherapeutic treatments (Paclitaxel, Vincristin, and Vinblastin) acting antimitotically could result in growth inhibition independent of the p53 status (81). Interestingly, the cells in one case with p53 mutation and with an increased chemosensitivity were treated with Paclitaxel (77).

However, the STS patients with non-frameshift mutations bearing tumors had an increased risk of dying because of their tumor, independent of irradiation and/or chemotherapeutic treatment. We suggest that such patients be excluded from p53-dependent therapies. The relationship of p53 mutations and prognosis, including a p53-independent therapy for these patients, needs to be studied prospectively.

In STS, additional gene alterations, especially in the *hmdm*-2 oncogene (human homologue to the mouse double minute gene), are important for tumorigenesis. Mdm2 interacts with p53 in an autoregulatory feedback loop (82,83). *Mdm*2 gene amplifications are observed in about 30% of sarcomas (84,85). Alternative-spliced mdm2 transcripts mostly missing the p53 binding site occur exclusively in human cancers, but not in normal tissues. Additionally, the frequency of these altered transcripts correlates with the tumor grade and stage (86). In our group of patients with STS we were able to show that an Mdm2 overexpression, detected immunohistochemically, predicts a very poor prognosis, dependent on and independent of p53 (87,88).

These results point to the necessity of considering molecular alterations in the multistep process of cancer, such as p53 mutations and mdm2 alterations, in future molecular genetic therapies for STS and other malignancies.

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