**mdm2 mRNA Level is a Prognostic Factor in Soft Tissue Sarcoma**

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**Abstract**

**Background:** The oncogenic properties of murine double minute-2 (mdm2) protein over-expression, which mostly results from the interaction with the tumor suppressor p53, are well described and their negative impacts on the prognosis of affected patients is well characterized. However, clinical relevance of mdm2 mRNA expression is poorly investigated.

**Materials and Methods:** In this study, 65 soft tissue sarcoma (STS) samples were analyzed for mdm2 mRNA expression by a quantitative reverse transcription polymerase chain reaction (RT-PCR) approach using available validated ready-to-use assays based on the TaqMan® technology (PE Applied Biosystems, Weiterstadt, Germany). Mdm2 data were correlated to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression calculated from the same sample.

**Results:** For patients with a mdm2/GAPDH mRNA ratio below 50 zmol/amol the survival was strikingly reduced in comparison to patients with a ratio of >50 (p = 0.0241). Multivariate Cox analysis showed that the difference in prognosis for patients with tumor stage 2 and 3 became even more pronounced between patients with a ratio of <50 zmol/amol and patients with a ratio of >50 (p = 0.0041; RR = 5.6).

To test if the group with an mdm2 mRNA expression >50 is homogenous concerning the prognosis, the group was divided into three subgroups with values of 0 to <100, 100 to <500 and ≥500. The subgroup with values of 100 to <500 showed the best prognosis (p = 0.0164); whereas, the one with values of 50 to <100 showed the worst prognosis in this group and, in between, was the one with values of >500. After omitting patients of stage 1 and 4, the subgroup with values of 100 to <500 showed an even more striking best prognosis (p = 0.0015); the other subgroups remained in the same sequence. The risk of tumor-related death over 5 years was most conspicuous in patients with mdm2 mRNA expression <50 than in those with ratios of 100 to <500 displaying a 13.3-fold higher risk. In a comparison between mdm2 mRNA levels and P53 protein expression or p53 mutational status, no relationship was found.

**Conclusions:** In our study, the mdm2 mRNA level appears to be an independent prognostic factor for STS patients, marking its role in STS genesis and as a potential factor for gene therapeutical approaches.

**Introduction**

The mdm2 gene was originally isolated by virtue of its amplification in a tumorigenic derivative of NIH-3T3 cells (1,2). Beside localiza-
tion of the human \textit{mdm2} gene to chromosome 12q13–14. \textit{mdm2} gene amplifications were detected in a variety of human malignancies and particularly in sarcomas (3–8). The oncogenic properties of the human \textit{mdm2} gene product have been attributed mostly to its interaction with the tumor suppressor gene \textit{p53} (9). \textit{Mdm2} promotes inactivation of \textit{p53} by its rapid degradation, inhibition of \textit{p53}-mediated apoptosis/growth arrest and by masking the transactivating domain of \textit{p53}, thus, impairing the interaction with the transcriptional machinery (3,10–19). The effect of \textit{mdm2} can be modulated by other proteins as RB-1 or p19 ARF (20,21). On the other hand, \textit{mdm2} can act independently from \textit{p53}, for example, it interacts with transcription factors of the \textit{E2F}-family and the human TATA-binding protein (22,23), inhibits RB growth regulatory function (24), contributes to tumorigenesis in \textit{p53}−/− mammary epithelial cells (25), mediates TGF-β1 resistance (26), and inhibits the G0/G1-S-phase transition in normal human diploid cells (27). In numerous tumor cell lines and malignant tumors, particularly sarcomas, the human \textit{MDM2} protein overexpression is a characteristic feature (28) which can be correlated to poor prognosis (7,29). However, overexpression may occur independently of gene amplification and might correlate with an increased transcription and/or a different translation efficiency of human \textit{mdm2} transcripts (30–35). Occurrence of different \textit{mdm2}-mRNA transcript levels and splice products in malignant tumors is well described (31,36,37), but only recently was an impact on tumor behavior and prognosis uncovered (38,39). Comparably less is known about \textit{mdm2} mRNA expression in sarcomas, beside correlating it to \textit{mdm2} gene amplification (37,39,40). Therefore, the aim of this study was to investigate the level of human \textit{mdm2} transcript, if there is a relationship to \textit{p53} mutational state or \textit{p53} protein expression, and if the \textit{mdm2} mRNA level has a prognostic impact for soft tissue sarcoma (STS) patients.

Materials and Methods

\textit{Tissue Specimens and Histopathological Data}

We examined 65 frozen tumor samples from 65 adult, non-selected soft tissue sarcoma STS patients (Institute of Pathology, University of Halle, Germany and Surgical Clinic 1, University of Leipzig, Germany); consisting of 18 malignant fibrous histiocytoma, 13 liposarcomas, 11 malignant neural tumors, 7 fibrosarcomas, 5 leiomyosarcomas, 4 rhabdomyosarcomas, 4 synovial sarcomas and 3 other STS. They comprised 44 primary tumors and 21 relapses. Tumors originated from different locations: at the extremities, 63%; intrabdominal/retroperitoneal, 23%; trunk wall, 9%; head/neck, 5%. Insofar as possible, surgical therapy for all patients was localization dependent: compartment resection, wide excision or multivisceral resection with tumor free resection margins for all samples confirmed in histological examination. Histoprognostic staging of the tumors showed: 5 (7.7%) stage 1; 33 (50.8%) stage 2; 19 (29.2%) stage 3; and 8 (12.3%) stage 4. Out of 65 STS patients, 25 (38%) died of the tumor after an average of 27 months (range 2 to 201); whereas, 40 (62%) of the patients are alive after an average observation period of 38 months (range 4 to 104).

\textit{RNA Preparation and \textit{cDNA} Synthesis from Clinical Samples and Cell Lines}

Ten to 20 cryosections (40 µm in thickness) of each STS sample were transferred to RNase-free 1.5 ml Eppendorf tubes and homogenized in 1 ml of “Reagent 14” (Integrated Separation Systems, Natick, MA). Whole RNA was isolated by running cycle program 805 using an Autogen 540 nucleic acid extraction robot (Integrated Separation Systems). cDNA was synthesized from 1 µg aliquots of purified, resuspended and ultraviolet (UV) absorption-measured RNA samples in a 20 µl standard reaction mixture containing AMV reverse transcriptase buffer (250 mM Tris/HCl, pH 8.3, 250 mM KCl, 50 mM MgCl2, 50 mM dithiothreitol, 2.5 mM spermidine), 5 U AMV reverse transcriptase, 0.5 mM of each dNTP (Promega, Madison, WI, U.S.A.), 10 U recombinant RNase inhibitor (AGS, Heidelberg, Germany), and 200 ng oligo(dT) (Amersham Pharmacia Biotech, Uppsala, Sweden) at 42°C for 1 hr. For this, a GeneAmp®9600 thermal cycler and 0.2 ml-MicroAmp® reaction tubes (PE Applied Biosystems, Weiterstadt, Germany) were used. RNA and cDNA samples were stored at −80°C until use.

\textit{Automated \textit{mdm2} and \textit{GAPDH} Transcript Analysis by Quantitative Fluorescence PCR}

Two commercially available, validated polymerase chain reaction (PCR) assays for quanti-
tation of murine double minute-2 (mdm2) and
glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene transcripts (ZeoQuant Nuk-
leinsaure-Diagnostika, Leipzig, Germany) were used in our laboratory (41). The mdm2 assay
detects all but the shortest splice variant of mdm2 mRNA, previously described by Sigalas
et al. (38). Briefly, conventional 96-well bases were loaded with an 8-well ready-to-use stan-
dard DNA strip either coated with eight differ-
ent amounts of reference-DNA for quantitation
mdm2 or GAPDH transcripts, respectively, for-
ward and reverse primer, and the TaqMan® probe. The double labeled probes were either 5’-labeled with the fluorescent reporter dye 6-
carboxyfluorescein (FAM) for detection of mdm2 or
2,7-dimethoxy-4,5-dichloro-6-carboxyfluoro-
rescine (JOE) for GAPDH, and the common 3’-
fluorescent quencher dye 6-carboxytetram-
ethylrhodamine (TAMRA) in order to generate
the respective mdm2 or GAPDH standard refer-
ence curves for each run. The remaining free
base positions were loaded with the required
number of sample tubes containing just the re-
spective TaqMan® oligonucleotide sets. Reac-
tion premixes containing PCR buffer, which
was supplemented with the passive fluores-
cence dye 6-carboxytetramethyl-rhodamin
(ROX), dNTPs, and 1.25 U of AmpliTaq®
(Gold) (PE Applied Biosystems) were assem-
bled according to the manufacturers instruc-
tions. Aliquots of the mixes were added to each
reaction tube by using a BIOMEK® 2000 labo-
ratory automation workstation (Beckman Instru-
mants Inc., Fullerton, CA, U.S.A.). Sample and standard reactions (final volume of 50 µl)
differed only by the addition of 2 µl aliquots of
the analyzed cDNA sample. PCR amplification
and detection was performed with an ABI
PRISM® 7700 Sequence Detection System (PE
Applied Biosystems). Sample cDNA amounts
were calculated from data obtained with the si-
multaneously amplified reference DNA strips.
Mdm-2 data were correlated to GAPDH cDNA
(zeptomoles [zmol, 10^-21] mdm2 mRNA per at-
tomole [amol, 10^-18] GAPDH mRNA) calcu-
lated from the same cDNA sample.

p53 Mutational Analysis

The tumor samples were examined for muta-
tions in the p53 gene by nonradioactive PCR-
SSCP-sequencing. DNA was isolated from
frozen tumor samples and the p53 gene (exons
4 to 9) was amplified in PCR reactions as de-
scribed previously (42). In an SSCP-pre-screen
for mutations, PCR products were investigated
in 6 or 10% (PAA)-ready-made gels (Novex,
Heidelberg, Germany) for abnormal single
strand DNA shifts and striking cases were cy-
cle-sequenced on an ABI 373 using the Dye
Terminator Kit (PE Applied Biosystems).

Western Blot Analysis

Thirty µg of total protein were separated on 10%
polyacrylamide/SDS gels (Mini gel system: Bio-
metra, Göttingen, Germany). Afterwards, pro-
teins were transferred to a PVDF Immobilon
membrane (Millipore; Eschborn, Germany) at
200 mA for 90 min (Miniblotter; Biometra). Af-
terwards, the membrane was blocked with 0.1%
Tween 20 containing 3% bovine serum albu-
min (BSA) and incubated for 1 hr with anti-pS3
antibody (DO-7; 1:500; Dianova, Hamburg,
Germany) or with anti-mdm2 antibody (1B10; 1:500;
L. Dox, Dossenheim, Germany) and 1 hr
with horseradish peroxidase-conjugated anti-
mouse immunoglobulin G (lgG) antibody
(1:1000; Dako, Denmark) at room temperature.
For protein detection, the membrane was placed
for 1 min in ECL-substrate (Amersham, Braun-
sweg, Germany) and exposed to Biomax
film (Kodak, Germany). The amount of P53
protein was compared with an internal posi-
tive control (p53 mutated RD cells/ATCC CCL
136 with a P53 over-expression) and standard-
ized to the β-actin band determined in the same
sample by densitometry (Imagemaster VDS
3.0; Pharmacia, Braunschweig, Germany). P53
and MDM2 expression were characterized ac-
cording to a semiquantitative scale as zero, mod-
est, marked, or strong expression. For the eval-
uation of the MDM2 protein expression, the
90kD, 85kD, 76kD, 74kD and 58kD bands
were considered as relevant.

Statistical Analysis

Prognostic analysis began with a descriptive
presentation of the cumulative survival func-
tions according to the Kaplan-Meier method
and a univariate evaluation of prognostic dif-
fferences with a log-rank test. The Cox regres-
sion model, which was used to estimate the ef-
fect of mdm2 mRNA expression on prognosis,
was adjusted to stage, localization and type
of surgical resection. A probability (p) of
< 0.05 was defined as significant and a relative
risk (RR) was calculated. The statistic analyses
were carried out using software from SPSS Inc. (SPSS 8.0). The cut points for \textit{mdm2} mRNA expression levels represent prognostic relevant thresholds, which were set after continuously sliding cut points in increment values of 10.

\textbf{Results}

\textit{mdm2} mRNA Expression

We investigated 65 STS samples for human \textit{mdm2} mRNA expression using the ABI PRISM® 7700 Sequence Detection System (PE Applied Biosystems) and quantitative ready-to-use fluorescence PCR assays. The assays, which were by automation using mostly robotic workstations supporting the 96-well format, allowed precise and reproducible data recovery combined with excellent sensitivity at low zeptomole detection levels. Dynamic ranges of usually 4–6 logs without prior sample dilution (up to $>10^8$ copies per tube) were easily achieved. The slopes of the individual reference curves were very close to the mean slopes (SD $\pm 2–5\%$), at simultaneous correlation coefficients of the calculated linear fits usually $>0.99$ (Fig. 1).

The correspondence of data with an in-house competitive GAPDH PCR protocol was found to be 99\% (41). Human \textit{mdm2} cDNA amounts were calculated from an external reference curve obtained with eight known amounts of \textit{mdm-2} standard reference DNA, calibrated as described earlier (43). Data were normalized to the number of GAPDH transcripts measured in the same cDNA sample, which were reported to be constant in several heterogenous tumors, tissues and cell lines (44–47). Thus, the calculated ratios of both cDNAs reflected the initial ratios of the mRNAs in the sample.

At first, a threshold for the ratio of \textit{mdm2} GAPDH-mRNA was determined after cut point sliding. A value of 50 (zmol \textit{mdm2}/amol GAPDH mRNA) as the most relevant cut point. In 15 STS samples, the ratio \textit{mdm2}/GAPDH-mRNA was below 50; whereas, the other 50 samples showed values $>50$, with a maximum of 7571. In a Kaplan-Meier curve, patients with \textit{mdm2} mRNA values below 50 showed a highly decreased average survival time (18 months), compared with the patients with \textit{mdm2} mRNA values $>50$ ($>60$ months). In an univariate log rank test, a significantly better survival for patients exhibiting \textit{mdm2} expression values $>50$ ($p = 0.0241$) was found.

For a multivariate analysis, a Cox regression model was applied to examine whether \textit{mdm2} mRNA expression was a prognostic factor independent of other known risk factors, such as tumor stage, kind of tumor resection and localization. As shown in Table 1, \textit{mdm2} mRNA expression ($<50$ versus $>50$) did not seem to be an independent prognostic factor ($p = 0.1355$). But after excluding patients with tumors of stage 1 and 4, the prognostic relevance became clearly visible ($p = 0.0041$, RR$=5.6$) (Table 1, Fig. 2). This can be reasoned by the fact that survival of both patient groups might be rather independent of \textit{mdm2} mRNA levels (i.e. patients with stage 1 tumors had mostly a good survival; 4 out of 5 patients with a ratio $>50$ survived); whereas, for patients with stage 4 tumors, poor prognosis is determined by the occurring manifested metastases. Patients with stage 2 and 3 tumors are of especially high interest in clinical practice, because their tumors may have a similar histo-morphological appearance; whereas, tumor behavior and prognosis can differ dramatically. When primary tumors and relapses were investigated, no difference in the \textit{mdm2} mRNA content was found (data not shown).

Next, we investigated if elevated human \textit{mdm2} transcript levels may be generally correlated with better survival or if this patient group might consist of several subpopulations with a diverging prognosis. When the group of patients with a \textit{mdm2}/GAPDH-mRNA ratio $>50$ was subdivided into three groups (50 to...
<100; 100 to <500; and ≥500; Table 2), the group with a ratio of 100 to <500 was distinguished by significantly better survival, compared with the reference group showing a ratio of <50 (p = 0.0164). The remaining two groups still showed a better survival than the reference group, but no statistical significance (p = 0.1981 and p = 0.2003) was found. Again, excluding patients with stage 1 and 4 tumors, the group with a ratio of mdm2/GAPDH-mRNA of 100 to <500 showed the best survival (76% of patients alive) at an increased significance level (p = 0.0015; Fig. 3), compared with the reference group (42% of patients alive). Most striking was that the risk of tumor-related death was 13.3-fold increased in the patients with a mdm2 mRNA expression <50, comparison with those with a ratio of 100 to <500 (Table 2).

P53 and MDM2 Protein Expression

P53 protein expression was detected by Western blots analysis and normalized to actin expression. A gross differentiation between four P53 expression groups divided into zero, modest, marked or strong expression was made. The group with zero and with strong expression showed the highest number of cases, which was not surprising since tumors may have lost p53 or may show abnormally high P53 protein expression levels. However, no relationship between the mdm2 RNA level and the detectable P53 protein expression, independent of considering tumors stage was observed (Table 3). In previous studies, a significant relationship between MDM2 protein expression, detectable immunohistochemistry, and a poor prognosis was found (29). However, the MDM2 expression, detected immunohistochemically or in Western blots, did not correlate with either the mdm2 mRNA level or survival (data not shown).

p53 Mutational Analysis

Fifty-five out of 65 STS samples were investigated for p53 mutations (exons 4-9). In eight

Table 1. Cox-Regression model (adjusted to tumor stage, kind of tumor resection and localization) for mdm2 mRNA expression and survival, after setting a threshold for mdm2 mRNA expression at a ratio of 50

| mdm2 mRNA Expression | Number of Samples per Tumor Stage | p    | RR  | Number of Samples per Tumor Stage 2 and 3 | p    | RR  |
|----------------------|-----------------------------------|------|-----|------------------------------------------|------|-----|
| <50                  | 0 6 6 3 15                        | *    | 2.2 | 0 16 36 3 15 * 2.2 12 * 5.6             |      |     |
| ≥50                  | 5 27 13 5 50                      | 0.1355 | *  | 5 13 15 5 50 0.0041 * 5.6             |      |     |
| total                | 6 33 19 9 65                      |      |     |                                           | 52   |     |

p = probability
RR = Relative risk
*reference group

Fig. 2 Multivariate Cox model for mdm2 mRNA expression and survival of STS patients (stage 2 and 3; n = 52). The threshold for the ratio of mdm2/GAPDH mRNA was set at a value of 50, after threshold sliding in Cox regression analyses. Curves for patients with an mdm2 mRNA level below 50 (-----) and >50 (…) are significantly different (p = 0.0041). A relative risk of 5.6 of tumor-related death is associated with a mdm2 mRNA level below 50, compared with a higher mdm2 mRNA expression level.
cases, a p53 mutation was detected as previously described (48). Independent of the type of mutation, all patients but one showed a higher mdm2 mRNA expression (>50), four cases were in the range of 50 to 100, and three in the range of 100 to <500. Concerning survival in the group with a mdm2 mRNA expression of 50 to <100, two out of four patients died and, in the group with 100 to <500, one out of three patients died (Table 4). This followed the observed trend that patients with mdm2 mRNA expression 100 to <500 had an increased overall survival. But we suggest, that rather the type of p53 mutation, i.e., patients with non-frameshift mutations had a poorer survival than those with frame-shift mutations, seems to correlate with prognosis (48).

**Discussion**

Our results show that human mdm2 mRNA expression, measured with an automated quantitative RT-PCR protocol, is an independent molecular prognostic factor for STS patients. In a Kaplan Meier test, the survival rate of patients with a $\text{mdm2/GAPDH-mRNA ratio}<50$ was strikingly reduced (average, 18 months survival time), compared with patients with a ratio $>50$ (average 60 months survival time) ($p = 0.0241$). In a multivariate Cox model confined to patients with tumor stage 2 and 3, the difference in prognosis became even more pronounced between patients with a ratio of $>50$ and patients with a ratio of $>500$ ($p = 0.0041$; RR = 5.6).

The result that diminished, rather than increased, human mdm2 mRNA expression correlates with a poor survival is somewhat surpising.

**Table 2. Cox-Regression model (adjusted to tumor stage, kind of tumor resection and localization) for mRNA expression and survival after subdividing patients in four groups according to mdm2 mRNA expression levels**

| mdm2 mRNA expression | Number of Samples per Tumor Stage 1 to 4 | $p$ | RR | Number of Samples per Tumor Stage 2 and 3 | $p$ | RR |
|----------------------|----------------------------------------|-----|----|------------------------------------------|-----|----|
| $<50$                | 15                                     | *   | 3.7| 12                                       | *   | 13.3|
| 50 to <100           | 14                                     | 0.1981 | 1.8| 11                                       | 0.2035 | 5.4|
| 100 to <500          | 26                                     | 0.0164 | *| 21                                       | 0.0015 | *|
| $>500$               | 10                                     | 0.2003 | 1.5| 8                                        | 0.0892 | 3.2|
| total                | 65                                     |     |    | 52                                       |     |    |

$p =$ probability  
RR = Relative risk  
$^*$stage 2: 33 patients, stage 3: 19 patients  
$^*$reference group
To our knowledge, only one group described a very similar observation made in ovarian carcinomas (49). Comparable with our data, a higher \textit{mdm2} mRNA expression was associated with a better prognosis in patients with ovarian carcinomas. On the other hand, there are several reports that describe significantly higher levels of \textit{mdm2} mRNA expression that are associated with an unfavourable prognosis in (AML) patients (30), with a subset of aggressive breast tumors (50) and with high invasiveness of hepatocellular carcinomas (51). Furthermore, it is well known that many malignant tumors, and particularly sarcomas, are characterized by elevated levels of \textit{mdm2} protein (28), which can be associated with a poor survival for sarcoma patients (7,29,52). However, our data suggest that a protein overexpression does not necessarily correlate with increased \textit{mdm2} mRNA expression. Therefore, it would be of high interest to find out if increased MDM2 protein expression is based on increased mRNA stabilization rather than on upregulation of transcription. There are several reports showing RNA stabilization as a major reason for increased mRNA levels, for example the heat shock protein hsp 70, insulin-like growth factor binding protein IGFB-BP3 and \textit{waf1} (53–55). Noticeably, the latter two genes are like \textit{mdm2} target genes of \textit{p53} and, for \textit{waf1}, mRNA stabilization, can be \textit{p53}-dependent and \textit{p53}-independent (53). We did not find any relationship between \textit{mdm2} mRNA level and \textit{p53} protein expression. However, the relationship between \textit{p53} protein level and \textit{mdm2} mRNA expression is controversial. On one hand, there are several reports showing no relationship (6,37,56,57). On the other hand, some studies concluded that tumors with \textit{p53} mutations may have an decreased \textit{mdm2} mRNA expression or that tumors without \textit{p53} mutations were characterized by higher \textit{mdm2} mRNA expression (40,51). In our study, seven out of eight tumor samples with

| \textbf{PS3 protein expression} | \textbf{mdm2 mRNA expression} | \textbf{mdm2 mRNA expression} |
|-------------------------------|-------------------------------|-------------------------------|
| \textbf{tumor samples stages 1 to 4} | \textbf{tumor samples stages 2 and 3} | \textbf{<50} | \textbf{50 to <100} | \textbf{100 to <500} | \textbf{\geq500} |
| none | 7 | 5 | 7 | 50 | 7 | 5 |
| moderate | 8 | 1 | 1 | 50 | 1 | 1 |
| marked | 9 | 1 | 2 | 50 | 2 | 1 |
| strong | 10 | 1 | 3 | 50 | 3 | 1 |
| total | 15 | 10 | 14 | 50 | 13 | 12 |

Table 4. Comparison of \textit{mdm2} mRNA expression and \textit{p53}-mutational status in relation to survival

| Case | \textit{p53}-mutational status | \textit{mdm2} mRNA expression | Survival/ observ. time (mos.) | \textbf{Survival} |
|------|-------------------------------|-------------------------------|-------------------------------|-----------------|
| S1/92 | non-fs-dp | 57.2 | 40 | a |
| M44 | ts | 78.5 | 6 | d |
| G25/92 | non-fs-del | 94.2 | 20 | d |
| M42 | non-fs-del | 230.9 | 24 | d |
| US8-93 | nonsense | 45.8 | 15 | d |
| G14-93 | fs-del | 70.3 | 31 | a |
| GS4-92 | fs-del | 138.5 | 39 | a |
| LS6 | fs-del | 193.3 | 46 | a |

Abbreviations: a, alive; d, dead; del, deletion; dp, duplication; fs, frameshift; non-fs, non-frameshift; observ., observational time; ts, transition.
p53 mutations did not show a striking decrease of mdm2 mRNA levels (50 to <100 and 100 to <500; Table 4). One possible explanation could be that one remaining wild type p53 allele is capable of maintaining mdm2 gene activity (58). Alternatively, mdm2 mRNA expression can occur independently from p53 (26,37,59). Furthermore, tumor cell lines with high levels of transcriptionally inactive p53 only might be unable to induce the expression of the MDM2 protein (60). Recently, a correlation between alternatively spliced mdm2 transcripts (missing the p53-binding site) and stabilized wild type p53 protein could be shown in glioblastoma cells, but its significance still remains unclear (61). Although we could detect short alternatively spliced forms of the mdm2 mRNA in STS, the correlation between the occurrence of spliced transcripts and the p53 gene status (wild-type/mutant) and the P53 protein expression level remains unclear (Bartel et al., submitted). However, the relationship between mdm2 mRNA level and P53 protein expression is not yet clear and needs further investigation (28). A comparable negative result was obtained by the attempt to correlate MDM2 protein expression previously evaluated by immunohistochemistry (52) or by Western blot analysis (data not shown) to mdm2 mRNA expression level. Furthermore, neither P53 nor MDM2 expression could be correlated to prognosis of the investigated 65 STS patients in a multivariate Cox regression analysis, which might be due to the relatively small number of patients.

Nevertheless, it seems necessary to stress here that only alterations in the gene, transcript or/and translational level may result in an oncogenic potential of mdm2. mdm2 normally acts as a cell cycle regulator (27), co-transcriptional factor (22,23) and a cellular regulator of several P53 protein functions (16,62,63). We suggest a target for gene therapeutical approaches.

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