TIMP-1 gene deficiency increases tumour cell sensitivity to chemotherapy-induced apoptosis

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The matrix metalloproteinases (MMPs) are a family of proteolytic enzymes, which are partly responsible for the turnover of the extracellular matrix in normal physiological conditions of tissue remodelling as well as in disease conditions such as invasion of cancer cells (Egeblad and Werb, 2002). Tissue inhibitor of metalloproteinases-1 (TIMP-1) is one of four inhibitors of the MMPs (Brew et al., 2000). It forms 1:1 stoichiometric complexes with the enzymes, thereby inhibiting the proteolytic activity of these molecules. Since the proteolytic activity of the MMPs is believed to facilitate invasion of cancer cells, one would expect TIMP-1 to inhibit tumour progression. However, a number of studies have demonstrated that the level of TIMP-1 is increased in several cancer forms, for example, colorectal and breast cancer and this increase has often been associated with a poor clinical outcome of the cancer patients (Ree et al., 1997; Holten-Andersen et al., 1999, 2000; McCarthy et al., 1999; Schrohl et al., 2004; Yukawa et al., 2004). This paradoxical finding has been suggested to be the consequence of distinct tumour-stimulating functions demonstrated for TIMP-1, for example, stimulation of proliferation (Hayakawa et al., 1992) and inhibition of apoptosis (Guedez et al., 1998; Li et al., 1999; Murphy et al., 2002; Lee et al., 2003, 2005; Boulday et al., 2004; Murphy et al., 2004).

The antiapoptotic effect of TIMP-1 induced by various apoptotic agents has been demonstrated in several different cell types such as human breast epithelial cells (Li et al., 1999; Liu et al., 2003, 2005), human breast carcinoma cells (Lee et al., 2003), human endothelial cells (Boulday et al., 2004), hepatic stellate cells (Murphy et al., 2002, 2004) and Burkitt’s lymphoma cells (Guedez et al., 1998). Of these studies, only Li et al. (1999) investigated the association between TIMP-1 level and cell survival following treatment with chemotherapy (adriamycin). However, a specific event of apoptosis was not confirmed in this survival experiment. In support of an antiapoptotic function of TIMP-1, our laboratory has recently shown that in patients with metastatic breast cancer, the response to chemotherapy was 0% in patients with primary tumours containing high levels of TIMP-1, while being 45% in patients with tumours containing low levels of TIMP-1 supporting a protective role of TIMP-1 to chemotherapy-induced apoptosis (Würtz et al., 2005). Similar results have also been demonstrated for metastatic colorectal cancer (Sørensen et al., 2006).

Taken together, this raises the hypothesis that TIMP-1 protects cells against chemotherapeutic treatment by inhibiting apoptosis. In order to investigate this hypothesis, we have established TIMP-1 gene deficient and TIMP-1 wild-type fibrosarcoma cell lines from lung tissue originating from littermate mice. We have confirmed these two different TIMP-1 variants by PCR, RT–PCR, Western blotting and ELISA. We have demonstrated that the cells display...
immortalised and clonogenic growth, showing that the cells had spontaneously transformed to a malignant phenotype. In addition, we have analysed the sensitivity of the cells to chemotherapy-induced apoptosis and shown that TIMP-1 gene-deficient cells are considerably more sensitive to induced apoptosis compared to the corresponding TIMP-1 wild-type cells. This confirms that TIMP-1 plays a role in inhibition of chemotherapy-induced apoptosis in the established cell lines. This cell system represents a powerful tool for further studies of the antiapoptotic role of TIMP-1 in cancer, especially considering the quality of having a model system consisting of gene deficient cells and their identically genetic wild-type control. Furthermore, this cell system can be used to uncover the mechanisms and signalling pathways involved in the TIMP-1-mediated inhibition of apoptosis. Evidence from a number of recent studies suggests that the focal adhesion kinase (FAK)/phosphatidylinositol-3 kinase (PI-3 kinase)/Akt/Bad/Bcl-X L/Bcl-2 signalling pathway is involved in the TIMP-1-mediated inhibition of apoptosis (Guedez et al., 1998; Li et al., 1999; Lee et al., 2003; Liu et al., 2003, 2005; Boulday et al., 2004); however, further studies are still needed to identify the mechanisms involved.

Importantly, as suggested by the present study, TIMP-1 may be a new therapeutic target in the optimisation of treatment with conventional anticancer drugs and future studies using this cell system offers the opportunity to investigate this hypothesis.

MATERIALS AND METHODS

Compounds

Etoposide (Bristol-Myers Squibb, Denmark), cytosar (Pfizer, Denmark) and vincristine (Faulding, Denmark) were kindly provided by Peter Buhi Jens (Rigshospitalet, Denmark).

Establishment of cell cultures from transgenic mice

TIMP-1 gene-deficient and wild-type mice were kindly provided by ML Davidsen, SØWürtz (Taconic Europe) all with negative results.

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Genotyping

The cells (passages 60–69) were trypanosed, harvested and DNA was isolated as described previously (Laird et al., 1991). One microlitre of each DNA sample was transferred to 25 μl PCR working solution (1 × Hot Star Tag Mastermix (Qiagen, Ballerup, Denmark), MgCl2 (25 mM) and 2 μl of each primer: forward primer recognising Twt: 5′-CAAGGGTGTTAGTGGCTTG-3′ (exon 3); reverse primer recognising Tko: 5′-GGCAAGGCGCATTGGACG-3′ (neo-cassette) and reverse primer recognising Tko: 5′-CATCGGGCCGCGCTAGACC-3′ (neo-cassette). The expected size of the Twt-specific PCR product is approximately 370 base pairs (bp), whereas the Tko-specific PCR product is approximately 670 bp. The reaction conditions optimised for the two genotype-specific bands were 95 °C for 15 min, followed by 32 cycles of 94 °C for 1 min, 63 °C for 1 min, 72 °C for 1 min and finally 72 °C for 10 min. In each assay, three controls were included: TIMP-1 +/0, TIMP-1 −/0 (TIMP-1 is located on the X-chromosome and only male mice were employed in the experiments; Huebner et al., 1986) and as negative control H2O. Tissue inhibitor of metalloproteinases-1 +/0 and TIMP-1 −/0 control samples were obtained by enzymatic digestion of tail tissue from TIMP-1 wild-type and TIMP-1 gene-deficient mice, respectively. The PCR products were run on a 2% agarose gel (Fermentas, Helsingborg, Sweden), stained with ethidium bromide and visualised by UV light.

RT-PCR

RNA was extracted from the cells (passages 72–78) using an SV Total RNA isolation kit (Promega, Manheim, Germany). The cells were grown in 10 cm culture dishes to a confluence of 90% and lysed in 500 μl lysis buffer. RNA from 175 μl of the lysed cells was extracted following the manufacturer’s instructions. DNase treatment was included in the protocol to avoid genomic DNA contamination of the RNA. Concentration of the RNA was estimated by measuring OD260. Two micrograms of RNA was reverse transcribed to cDNA in 20 μl RT buffer (Fermentas) containing 1 μl dNTPs, 20 μl Ribolock RNase inhibitor, 0.5 μg oligo(dT) primer, 0.2 μg random hexamer primer and 40 μM-M-MuLV Reverse Transcriptase. Samples were incubated at 25 °C for 10 min, followed by 42 °C for 1 h. The reaction was terminated by incubation at 95 °C for 5 min followed by cooling on ice. To ensure that RNA extractions and RT reactions were successful, PCR using introspanning β-actin primers was performed on all cDNA samples (forward primer: 5′-CGGTGGGCGGCCTAGGCACCA-3′ and reverse primer: 5′-TGGCCTATTAGGCTTGGGAG-3′). The expected size of the β-actin PCR product was 242 bp. To test for expression of TIMP-1 mRNA from Twt and Tko cells, PCR using introspanning TIMP-1-specific primers was carried out on cell cDNA (forward primer: 5′-GGTGGGAAATGCGCGGAGTATC-3′ and reverse primer: 5′-GAGCTGTACAGTCCCAAAC-3′). The expected size of this PCR product is 292 bp. PCR was performed in 25 μl reactions consisting of 1 × Hot Star Taq Mastermix, 1 μM of each gene-specific primer and 1 μl cDNA. Reaction conditions were 95 °C for 1 min followed by 40 cycles of 94 °C for 1 min, 60 °C for 30 s and 72 °C for 1 min. PCR products were run on a 1.5% agarose gel (Fermentas), stained with ethidium bromide and visualised by UV light.

Western blotting

Cells were seeded in 10 cm Petri dishes in CM. When about 90% confluent, the cells were harvested by scraping and resuspended in
100 µl lysis buffer (0.5% Triton X-100, 25 mM Hepes, 5 mM MgCl₂, 1 mM EGTA, milliQ H₂O) containing four protease inhibitors (Aprotinin, Leupeptin, Pepstatin A and Pefablock) in a final concentration of 1 µg/ml. The protein concentration in each sample was determined by protein BCA Protein Assay Kit (Pierce, Rockford, IL, USA). Proteins were separated by sodium dodecyl sulphate (SDS)–gel electrophoresis using a 12% polyacrylamide gel and blotted onto nitrocellulose paper. The blot was blocked in washing buffer (phosphate-buffered saline (PBS) + 0.1% Tween 20) containing 5% dry milk and incubated with the rat-IgG antibody to murine TIMP-1 (R&D Systems, UK, 5 µg/ml in washing buffer containing 1% dry milk) or the goat-IgG antibody to murine p53 (R&D Systems, diluted 1:2000 in washing buffer containing 1% dry milk) or rat-IgG antibody to murine p19ARF (Upstate, Charlottesville, USA, diluted 1:100 in washing buffer containing 1% dry milk) for TIMP-1, p53 and p19ARF detection, respectively. Subsequently, the blot was washed 3 x 10 min in washing buffer followed by incubation with the appropriate horseradish peroxidase-conjugated secondary antibody (rabbit-IgG to rat-IgG diluted 1:1000 or rabbit-IgG to goat-IgG diluted 1:10 000 or rabbit-IgG to rat-IgG diluted 1:2000 (DAKO, Denmark), respectively). Following 3 x 10 min washes in washing buffer, the blot was developed by the ECL detection system (Amersham Bioscience, UK) according to the manufacturer's instructions. In order to obtain a loading control, the blot was stripped and re-probed with a primary monoclonal antibody recognising glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Biogenesis, UK, diluted 1:80 000 in washing buffer containing 1% dry milk). Subsequently, the blot was washed 3 x 10 min in washing buffer and incubated with a polyclonal horseradish peroxidase-conjugated goat-IgG to mouse-IgG (DAKO, Denmark, diluted 1:100 000 in washing buffer containing 1% dry milk) for 1 h. Finally, the blot was washed 3 x 10 min in washing buffer and developed as described above. Details regarding cells are indicated in the figure legends.

Murine TIMP-1 ELISA

Cells (2500 cells/well, passages 55–56) were seeded in 96-well microtitre plates in triplicate in 100 µl CM. After 24 h, the CM was replaced with 150 µl new CM. After another 24 h, the CM was collected, centrifuged for 5 min at 300 g and the supernatant transferred to Eppendorf tubes and stored at −20°C. The amount of TIMP-1 secreted in the CM was measured using Quantikine Mouse TIMP-1 Immunoassay (R&D Systems, UK) according to the manufacturer's instructions.

Clonogenic assay

Clonogenic assay was performed as described previously (Jensen et al., 1993). In brief, cells (passages 27–35) were suspended in CM supplemented with penicillin (50 U/ml) and streptomycin (50 µg/ml). A volume of 350 µl cell suspension was mixed with a mixture of agar and medium. One microlitre of the cell-containing agar was then plated in triplicate in Petri dishes over a layer of sheep red blood cells. When the agar had solidified, 1 ml of medium were added on top. Cells were grown in a CO₂ incubator (7.5% CO₂) at 37°C and 100% humidity. Following 3 weeks of incubation, colonies (>64 cells) were counted using the software Sorcerer (Perceptive Instruments, Suffolk, UK).

Flow cytometrical analysis

DNA ploidy of early (passages 29–38), middle (passages 45–48) and late passages (passages 62–71) was determined for each cell line by flow cytometrical analysis as described previously (Vindelov and Christensen, 1990; Romer et al., 2005). All measurements were related to the DNA index of normal diploid mouse cells.

Cell death assays

Lactate dehydrogenase (LDH) release assay. To investigate the response to chemotherapy in the cell lines, cytotoxicity was determined following treatment with chemotherapeutic drugs. During cell culture conditions, cells that have been given an apoptotic stimulus will initially die by apoptosis and later turn into secondary necrosis due to the lack of phagocytosis. Cytotoxicity or cell lysis can be measured by the release of LDH in the culture supernatant. To measure the LDH release, the Cytotoxicity Detection Kit (Roche, Hvidovre, Denmark) was employed. The cells (passages 39–87) were seeded in 96-well microtitre plates (3000 cells/well). After 24 h, the cells were treated with the chemotherapeutic drug. After 48 h (etoposide) or 35 h (cytosar and vincristine) incubation, 50 µl (out of 200 µl) of culture supernatant was transferred to a new 96-well microtitre plate and mixed with 50 µl of substrate mix. The remaining culture supernatant was discarded and the residual intact adherent cells were lysed by the addition of 200 µl lysis buffer (1% Triton X-100 in CM). Following lysis for 30 min at 5% CO₂ and 37°C, 50 µl of the lysate was transferred to a new 96-well microtitre plate and mixed with 50 µl of substrate mix. Both the cell culture supernatants and the lysates were incubated with substrate mix for 10 min at room temperature protected from light. The absorbance was measured in a spectrophotometer at λ₂₆₃₀ nm and reference λ₂₆₃₀ nm. The amount of released LDH in per cent was related to the total amount as follows:

\[
\text{Cytotoxicity} \, (\% \text{LDH release}) = \frac{(LDH_{\text{supernatant}}/Total \text{LDH} \times (LDH_{\text{supernatant}} + LDH_{\text{lysate}}))\times 100\%}{LDH_{\text{supernatant}}}
\]

Apoptosis assay. To confirm that the cell death observed in the LDH assay was apoptotic, the presence of DNA–histone complexes in the cytoplasm following the apoptotic stimuli was measured. For this purpose, the Cell Death Detection ELISA Kit (Roche) was employed. The cells (passages 39–87) were seeded in a 96-well microtitre plate (3000 cells/well). After 24 h, the cells were treated with the chemotherapeutic drug. After 48 h (etoposide) or 35 h (cytosar and vincristine) and the level of apoptosis was measured according to the manufacturer’s instructions. The degree of apoptosis (DNA fragmentation) was calculated as follows: absorbance of sample (dying and dead cells)/absorbance of corresponding control (viable cells).

Cell proliferation assay

Cell proliferation was estimated using the CyQuant® Cell Proliferation Assay Kit (Molecular Probes Inc., Eugene, OR, USA/Invitrogen, Denmark). Cells (500 cells/well, passages 49–51 and passages 81–86) were seeded in 96-well microtitre plates and harvested daily over a 5- or 6-day period. Proliferation rates were analysed by spectrophotometrical measurements of the DNA and RNA content at 480 nm excitation and 520 nm emission according to manufacturer's instructions.

RESULTS

TIMP-1 genotype and TIMP-1 expression

In order to confirm the absence or presence of TIMP-1 in the TIMP-1 gene-deficient and the TIMP-1 wild-type cells, respectively, genotyping, RT–PCR, Western blotting and ELISA were performed (Figure 1). Genotypes of the TIMP-1 wild-type and TIMP-1 gene-deficient cells were determined by PCR (Figure 1A). As expected, the wild-type control (TIMP-1 + /0) gave rise to a
PCR product of approximately 370 bp representing the wild-type TIMP-1 gene (Figure 1A, lane 5). Bands of the same size appeared when PCR was performed on lysates from the Twt-II and Twt-III cell lines, thereby confirming that both of these cell lines exhibit the wild-type TIMP-1 gene (Figure 1A, lane 3). In contrast, when PCR was performed on lysates from the wild-type cells, each sample with each control with both sets of primers. (B) RT-PCR. Lane 1: Twt-II in passage 73; lane 2: Twt-III in passage 78; lane 3: Tko in passage 72; and lane 4: Tko-III in passage 78. The RT-PCR has been run on lysate from the cells, each sample with intron-spanning β-actin primers and with intron-spanning TIMP-1-specific primers. (C) Western blotting. Lane 1: Tko-II in passage 74; lane 2: Twt-II in passage 68; lane 3: Tko-III in passage 74; and lane 4: Twt-III in passage 70. In each lane, equal amounts of protein (60 μg protein) are loaded. The presence of two bands may reflect two different glycosylation products of TIMP-1.

**Figure 1** Confirming the absence or presence of TIMP-1 in the gene-deficient and wild-type fibrosarcoma cell lines respectively. (A) Genotyping. Lane 1: Twt-II in passage 60; lane 2: Tko-II in passage 60; lane 3: Twt-III in passage 65; lane 4: Tko-III in passage 65; lane 5: +/+ TIMP-1 control; and lane 6: −/− TIMP-1 control. The PCR was performed on lysate from the cells, each sample with each control with both sets of primers. (B) RT-PCR. Lane 1: Twt-II in passage 73; lane 2: Twt-III in passage 78; lane 3: Tko in passage 72; and lane 4: Tko-III in passage 78. The RT-PCR has been run on lysate from the cells, each sample with intron-spanning β-actin primers and with intron-spanning TIMP-1-specific primers. (C) Western blotting. Lane 1: Tko-II in passage 74; lane 2: Twt-II in passage 68; lane 3: Tko-III in passage 74; and lane 4: Twt-III in passage 70. In each lane, equal amounts of protein (60 μg protein) are loaded. The presence of two bands may reflect two different glycosylation products of TIMP-1.

**Figure 2** Transformation of the cell lines. (A, B) DNA ploidy analysis of Twt-II and Tko-II and Twt-III and Tko-III, respectively, in early, middle and late passages. A DNA index of 1 represents normal diploid mouse DNA content. (C) Western blot of p53 expression in the fibrosarcoma cells. Lane 1: Tko-II in passage 11; lane 2: Tko-II in passage 69; lane 3: Twt-II in passage 68; lane 5: Tko-III in passage 12; lane 6: Tko-III in passage 74; lane 7: Twt-III in passage 10; and lane 8: Twt-III in passage 70. In each lane, equal amounts of protein (40 μg) are loaded.

**Transformation of the cells**

The cells were analysed to establish whether they had spontaneously transformed to a malignant phenotype. All four cell lines displayed immortalised growth since they survived in culture at least to passage 120. More importantly, the clonogenic assay demonstrated that all cell lines were able to form colonies in soft agar (data not shown). Loss of euploidy is a characteristic of the transformation process and therefore we investigated DNA ploidy of the cells (Figure 2A and B). This analysis showed that both Twt-II and Tko-II cells were aneuploid in all the investigated passages according to their DNA indices of approximately 1.5. Both cell lines had subpopulations in different passages (Figure 2A). Likewise, the Tko-III cells were aneuploid, having a DNA index of approximately 1.6 with a single subpopulation. In contrast, the Twt-III cells were diploid with a DNA index of 1 in all passages tested (Figure 2B). Disruption of the p53 signalling pathway is also a frequent event in the transformation process. This typically occurs by an inactivation mutation in the p53 gene or inactivation of the tumour suppressor p19ARF, a positive regulator of the p53 gene. Inactivation of p53 directly leads to an increase in the expression level of the protein (Sousa and
Western blotting showed a pronounced increase in the expression level of p53 in late passages (> passage 68) compared to early passages (< passage 14) of Twt-II, Tko-II and Tko-III cells (Figure 2C). In contrast, the immunoblot analysis of the Twt-III cells revealed no detectable levels of p53 in either early or late passages. Thus, this further supports that the Twt-II, Tko-II and Tko-III cells have been malignantly transformed. The fact that there was no indication of inactivation of the p53 pathway in the Twt-III cells and that the cells were diploid implies that a different transformation process had taken place in this cell line. To analyse this further, we investigated the level of the tumour suppressor p19Arf. As determined by Western blotting, there was no expression of p19Arf in late passages of Twt-III cells, indicating that an indirect disruption of the p53 signalling pathway had taken place, thereby supporting that these cells had been malignantly transformed, although through a different process compared to the other cell lines (data not shown).

**TIMP-1 gene deficiency confers increased sensitivity to chemotherapeutic drugs**

TIMP-1 has been demonstrated to inhibit apoptosis induced by various stimuli in several different types of cells (Guedez et al., 1998; Li et al., 1999; Murphy et al., 2002; Lee et al., 2003; Liu et al., 2003, 2005; Boulday et al., 2004; Murphy et al., 2004). In order to investigate whether TIMP-1 inhibits chemotherapy-induced apoptosis, we tested the sensitivity of the TIMP-1 wild-type and the TIMP-1 gene-deficient cells to treatment with three different chemotherapeutic drugs and the cytotoxicity was measured by LDH release assay (Figure 3). In Figure 3A, the results from treatment of the Twt-II and Tko-II cell lines with the drug etoposide are shown. As seen in the figure, both cell lines displayed a dose-dependent response to the treatment; however, the TIMP-1 gene-deficient cells were considerably more sensitive to the treatment compared to the wild-type cells. To confirm that the cell death induced by etoposide was apoptotic, we determined the level of DNA–histone complexes in the cytoplasm following drug treatment. As is evident from Figure 3B, the cell death induced by etoposide was indeed apoptotic. The experiments with etoposide were also conducted with Twt-III and Tko-III with the same results (data not shown) showing that the TIMP-1-mediated protection was not dependent on the transformation process. We also tested the effect of the two chemotherapeutic drugs, cytosar and vincristine, on the Twt-II and Tko-II cell lines. The results from the LDH release assay as well as the apoptosis assay are shown in Figure 3C–F. As was the case concerning etoposide, the Tko-II cells were considerably more sensitive to the drugs tested compared to the Twt-II cells as determined by LDH release assay (Figure 3C and E). In addition, the apoptosis assay confirmed that the enhanced cell death observed in the Tko-II cells was a result of increased apoptosis (Figure 3D and F). Together, these results demonstrate that TIMP-1 deficiency increases the cellular response to chemotherapy-induced apoptosis.

**Similar growth rates of the TIMP-1 gene-deficient and wild-type cells**

In general, chemotherapeutic drugs induce apoptosis in cancer cells by disturbance of cell division through damaging of the DNA. Therefore, rapidly dividing cells are affected more by chemotherapy than normally dividing cells. To ensure that the differential response to chemotherapy observed in the cell lines is not the
result of different growth rates, we investigated the doubling times of the cells using a cell proliferation assay (Figure 4). In passages 49–51, the doubling time for Twt-II was 1.96 days and for Tko-II 1.86 days (Figure 4A), and in passages 81–86, the doubling times was 1.20 and 0.94 days (Figure 4B), respectively, confirming similar proliferation rates of the TIMP-1 gene-deficient and wild-type cells. Thus, the difference in sensitivity to induced apoptosis cannot be ascribed to a difference in growth rates. Similar growth rates were also found for Twt-III (passages 49–51: 1.28 days; passage 81–86: 1.21 days) and Tko-III (passages 49–51: 1.94; passage 81–86: 1.41 days).

**DISCUSSION**

It is well established that TIMP-1 is able to inhibit apoptosis induced by various apoptotic stimuli in a number of different cell types. A single study has suggested that TIMP-1 enhances survival during chemotherapeutic treatment (Li et al., 1999). In addition, recent clinical studies demonstrated a significant association between high tumour tissue levels of TIMP-1 and no objective responses to the most frequently used chemotherapeutic drugs for patients with metastatic breast cancer and colorectal cancer, indicating that TIMP-1 also protects against chemotherapy-induced apoptosis in vivo (Würtz et al., 2005; Sørensen et al., 2006).

In an attempt to obtain a system, which can be used for further investigations of the protective function of TIMP-1 against chemotherapy-induced apoptosis, we have established TIMP-1 wild-type and TIMP-1 gene-deficient fibrosarcoma cell lines from lung tissue originating from littermate mice. We have characterised these cells and confirmed the two different TIMP-1 variants by PCR, RT-PCR, Western blotting and ELISA. Moreover, we have assured that the cells have been malignantly transformed by demonstration of immortalised and clonogenic growth as well as aneuploidy and/or disruption of the p53 signalling pathway. It should be emphasised that this new well-characterised cell system has the strength of exhibiting two pairs of cell lines sharing, except from TIMP-1, identical genetic backgrounds, making it possible to investigate the isolated function of TIMP-1.

To investigate whether TIMP-1 could play a role in inhibition of chemotherapy-induced apoptosis in our cell system, we tested the response of the cells to apoptosis induced by three different chemotherapeutic drugs. We showed that the TIMP-1 gene-deficient fibrosarcoma cells were considerably more sensitive to chemotherapy than the corresponding TIMP-1 wild-type cells, confirming that TIMP-1 protects against chemotherapy in our model system. Interestingly, the experiments performed suggest that TIMP-1 inhibitors could represent a novel treatment as a chemosensitising approach given before conventional therapy and the model system presented here offers the opportunity to investigate this hypothesis.

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