Treatment of Colorectal Liver Metastases by Adenoviral Transfer of Tissue Inhibitor of Metalloproteinases-2 into the Liver Tissue

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ABSTRACT

Metastatic disease is the leading cause of death in cancer patients. Here, we describe a novel gene therapeutic strategy for prevention of metastatic spread by providing a suitable defense mechanism for the target organ. The production of metalloproteinase (MMP) enzymes by cancer cells is critical for local invasion and for infiltration of metastatic cells into distant sites. Using a nude mouse model of colorectal liver metastasis, we have overexpressed the MMP inhibitor, tissue inhibitor of MMP-2 (TIMP-2) in the liver prior to, or following, tumor challenge by metastatic LS174T cells in vivo. Transduction of ~50% of hepatocytes resulted in 95% reduction in metastasis after tumor challenge compared with controls. Furthermore, TIMP-2 gene transfer into livers with preexisting metastatic spread resulted in a 77% reduction in tumor cell growth. Our data imply that MMP activity of metastatic cancer cells is an absolute requirement for spread and subsequent tumor growth and that enhancing antiproteolytic defense mechanisms in target organs represents a novel form of cancer gene therapy.

INTRODUCTION

Colorectal carcinoma is the second most frequent cancer disease in both sexes (1). For patients with this type of cancer, liver metastases are the main cause of death. They often remain only the manifestation of the disease once the primary tumor has been surgically removed (2, 3). Therefore, they are a very attractive target for conventional therapeutic approaches. Because of the multiplicity and low accessibility of the metastases, surgical resection is, unfortunately, only possible for a minority of patients (2, 3). Systemic and regional chemotherapy has led to temporary remissions, but overall survival has not improved (2, 3). Therefore, new therapeutic approaches are urgently needed.

The metastatic spread of a primary tumor to distant organs involves a series of events that include the detachment of the cells from the primary tumor, migration and invasion through the basement membranes of blood and lymph vessels, adhesion to the endothelium of secondary organs, and extravasation and intravasation of the secondary organ. Metastatic tumor cells are known to produce a variety of proteolytic enzymes that are required to degrade ECMs,2 promoting intravasation and extravasation. Among these are matrix MMPs, a family of at least 20 zinc-dependent endopeptidases that collectively have the capacity to degrade all of the major components of the ECM (4) and that have been extensively implicated in cancer progression (5–7). Moreover, MMPs appear to be involved in the process of angiogenesis, mediating the remodeling and penetration of the ECM by new capillaries (8). Once, they are activated, MMPs are regulated by a group of specific endogenous inhibitors known as TIMPs. Under normal circumstances, such as tissue remodeling during embryogenesis, there is a tight regulation of MMP activity that is lost upon tumor growth and metastasis, leading to deregulated MMP activity in many solid tumors (7). Several studies have demonstrated elevated levels of MMP-1, MMP-2, MMP-9, and MMP-11 in colorectal cancer (9–13), suggesting a role for MMPs in this type of disease. MMP-2, the M, 72,000 gelatinase A that degrades substrates including gelatins, fibronectins, and type IV collagens (4), is among the best characterized of the MMPs. The enzyme is specifically inhibited by TIMP-2 (5, 14–16). TIMP-2 has been demonstrated to reduce tumor cell growth as well as metastasis (17, 18). Overexpression of TIMP-2 reduced primary tumor growth as well as hematogenous metastasis of transformed rat embryo fibroblasts (17, 18) and primary tumor growth but not metastases of metastatic human melanoma (19). In these studies, the general problem of efficient in vivo gene transfer into tumors was not addressed explicitly because TIMP-2 was transferred in vitro, or the vectors were preincubated with tumor cells before tumor induction. Conversely, in this study, we show for the first time that efficient in vivo transfer of TIMP-2 is possible by targeting the nontumorous tissue of a target organ, which circumvents the need to efficiently transduce the tumorous deposits themselves. We evaluated the potential of adenovirus-mediated overexpression of TIMP-2 to protect mouse livers from metastasis from a highly invasive cell line of colorectal origin (LS174T cells). We show that expression of TIMP-2 by hepatocytes protects from challenge by metastatic cells and, furthermore, reduces the growth of well-established metastases.

MATERIALS AND METHODS

Production of Adenoviruses, Cell Lines, and Animals. Construction of Ad-TIMP-2 has been described (20). Ad-bgal was generously provided by M. Perricaudet, (Institut Gustave Roussy, Villejuif, France, 21) and was propagated as described (22). For titering, we apply a modified end point cytopathic effect assay as described before (22). Briefly, 10 days after infection of 293 cells in 96-well plates, cells are scaped and seeded onto 24-well plates. Cells are grown for 7 days and then examined for plaques. This assay seems to give higher titers than the traditional cytopathic effect or plaque assays. LS174T and SW1116 human colon adenocarcinoma cell lines, the HuH7 human hepatocellular carcinoma cell line, and A2 hepatocytes derived from p53 knockout mice (23, 24) were cultured in DMEM (LS174T cells in RPMI) supplemented with 10% FCS, 2 mM glutamine, 100 IU/ml penicillin, and 50 μg/ml streptomycin. Female, athymic nude mice (NMRI-nu/nu) were purchased from M&B laboratories (Ry, Denmark).

Western Blotting for Quantification of Secretion of Recombinant TIMP-2. A2 cells were infected in DMEM without serum for 1.5 h with viruses and MOI as indicated. Supernatants were collected after 24 and 48 h and centrifuged for 5 min, and 10 μl were electrophoresed on 12% polyacrylamide/SDS gels. After transfer onto nitrocellulose membranes, TIMP-2 was detected with a mouse monoclonal antibody against human TIMP-2 (T2–101, Ab-1; Dianova, Hamburg, Germany), biotinylated goat antimouse antibody, and streptavidine-POD conjugate, followed by enhanced chemiluminescence.

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The abbreviations used are: ECM, extracellular matrix; MMP, metalloproteinase; TIMP, tissue inhibitor of MMP; MOI, multiplicities of infection; Ad-TIMP-2, Adeno-TIMP-2; Ad-bgal, Adeno-β-galactosidase; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; TUNEL, terminal deoxynucleotidyl transferase-mediated nick end labeling; pfu, plaque-forming unit(s).

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Detection of the Secretion of Recombinant TIMP-2 by Reversed Gelatin Zymography. Production of TIMP-2 by infected A2 cells was assessed by reversed gelatin zymography as described (20). Briefly, A2 cells were infected in DMEM without serum for 1.5 h with viruses and MOI as indicated. Supernatants were collected after 48 h, centrifuged for 10 min, and concentrated using Amicon filters (Lexington, MA). NaN₃, Brij-35, and CaCl₂ were added to 0.1%, 0.05%, and 5 mM, respectively. Samples were mixed with nonreducing buffer and loaded onto 10% polyacrylamide/SDS gels impregnated with 1 mg/ml gelatin (porcine skin type I, bloom 300, G2500; Sigma, Deisenhofen, Germany) and 107 ng/ml active MMP-2 protein (Oncogene, Cambridge, MA). After electrophoresis, SDS was removed by incubating for 2 h in 2.5% Triton X-100. The gel was then incubated overnight in 50 mM Tris-HCl (pH 8.0), 50 mM NaCl, 10 mM CaCl₂, 0.05% Brij-35, and 0.02% NaN₃ at 37°C. Gels were stained with 0.5% Coomassie Brilliant Blue (R250; Sigma), and bands of gelatinase inhibitory activity representing TIMPs appeared dark against the digested background.

Detection of Endogenous MMP-2 by Gelatin Zymography. Production of MMP-2 by tumor cells was assessed by gelatin zymography as described (20). Briefly, subconfluent cells were grown for 48 h in DMEM without serum. The supernatant was removed and centrifuged for 10 min at 1500 × g. If necessary, supernatant was concentrated using Amicon filters. Gels (7.5%, but without addition of activated MMP-2) were electrophoresed and incubated as for reversed zymography. Bands of lysis representing gelatinase activity were visualized against a dark background.

Detection of Recombinant Human TIMP-2 in the Serum of Nude Mice by ELISA. Adenoviruses were administered i.v. into the tail vein of nude mice. At various time points, blood was drawn, allowed to coagulate, and centrifuged for 10 min at 15,000 × g, and serum was collected. Samples were analyzed for TIMP-2 levels at three different dilutions using a quantitative ELISA assay (RPN 2618; Amersham Buchler, Braunschweig, Germany).

Immunohistochemistry and X-gal Staining. Adenoviruses were administered i.v. into the tail vein of nude mice. After 3 days, liver sections were snap frozen in liquid nitrogen. For immunohistochemical detection of TIMP-2 in liver tissue, a mouse monoclonal antibody against human TIMP-2 (1:10; T2–101, Ab-1; Dianova), followed by an FITC-conjugated sheep antimouse as the secondary antibody, was used. No staining was seen if an unspecific isotype antibody was used instead of the TIMP-2 antibody. Livers from mice infected with Ad-βgal were also stained with X-gal according to standard procedures.

For determination of angiogenesis, proliferation, and apoptosis, paraffin sections from the livers of both efficacy experiments (preventive and therapeutic approach) were deparaffinized and stained with the respective antibo-
was returned to the abdominal cavity, the peritoneum was closed by suture, and
the skin was closed with wound clips. For the time sequence of virus and tumor
cell applications, see the figure legends. Five weeks after tumor cell inocula-
tion (which had been determined previously as the week when 50% of the
animals had to be euthanized because of their tumor burden) was used as the
end point of experiments. Animals that had to be euthanized prematurely
because of tumor burden were also included into data acquisition.

The weight of liver metastases was determined as follows. The total mass
of metastatic liver tissue was measured, and for each animal, the percentage of tumor
mass versus normal liver tissue was quantified macroscopically. Then, the
livers were examined histologically in a blinded fashion by preparation of
leveled sections. To this extent, livers were fixed in 4% neutral-buffered
formalin and after thin sectioning were completely embedded in paraffin. One
to 2-μm thick sections were prepared from each block and were stained with
H&E. The percentage of tumor mass as compared with normal liver tissue was
estimated for all liver pieces and slides, and the mean for each liver was
calculated. The macroscopic and histological determinations of the tumorous
fraction were performed independently and were within a 10% deviation
between the methods. The mean of the two values was then taken. This value,
expressed as a decimal, was multiplied with the weight of the tumorous livers,
and the result was determined to be the actual tumor weight. For statistical
analysis, the Mann-Whitney test was applied.

For survival experiments, a dose of 0.5 × 10^6 LS174 cells was used.
Animals were euthanized when they appeared moribund. For statistical anal-
ysis, the Kaplan-Meier log rank test was applied.

RESULTS

Secretion of MMPs by Metastatic LS174T Cells. We first established an animal model for liver metastases. To achieve this, we used a model described previously in which tumor cells are injected into the spleen of nude mice, resulting in extensive liver metastasis (25). Among a panel of several colon carcinoma cell lines, LS174T cells have been shown to secrete large amounts of MMPs and to be highly tumorigenic (25). We therefore initially assessed MMP activity secreted from metastatic LS174T cells and other cell lines (Fig. 1). Gelatin zymography revealed that LS174T cells produced high levels of gelatinolytic MMP activity compared with other cell types, and this was predominantly MMP-2. We therefore used the LS174T cell line for in vivo analysis.

Adenoviral Transfer of TIMP-2 in Vitro and in Vivo. We first evaluated expression of TIMP-2 after adenoviral gene transfer in vitro. A2 cells were transduced with Ad-TIMP-2 at MOI 100 or 400, which leads to high-level gene transduction in this cell type (23). Among a panel of several colon carcinoma cell lines, LS174T cells have been shown to secrete large amounts of MMPs and to be highly tumorigenic (25). TIMP-2 (M_t ~21,500) was detected by Western blotting in the supernatants of Ad-TIMP-2-transduced cells but not in Ad-βgal or PBS-treated cells (Fig. 2a). No significant effect on cell survival was seen after infection at MOI 100 or 400 compared with control adenovirus-infected cells (data not shown).

We then demonstrated the functionality of TIMP-2 protein by reversed zymography (Fig. 2b). As expected, bands of MMP-2 inhibitory activity representing TIMP-2 were detected in the supernatants of Ad-TIMP-2-infected A2 cells but not in the supernatants of uninfected or Ad-βgal-infected cells.

We next evaluated adenovirus-mediated TIMP-2 gene transfer into mouse livers in vivo. Mouse livers have been shown to be highly susceptible to transduction by adenoviruses, even after systemic delivery of the vectors (26–28). For transduction of 50 and 100% of hepatocytes, 3 × 10^10 or 6 × 10^10 pfu were, respectively, sufficient (Fig. 3, B and C) as determined by X-gal staining 3 days after delivery of Ad-βgal.

Similar efficacies of gene transfer could be detected immunohistochemically with an antihuman TIMP-2 antibody when 3 × 10^10 pfu or 6 × 10^10 pfu Ad-TIMP-2 were injected (Fig. 3, E and F). No TIMP-2 immunoreactivity was observed in the uninfected (Fig. 3D) or Ad-
TIMP-2 GENE THERAPY FOR LIVER METASTASES

βgal-infected (not shown) controls. TIMP-2 immunoreactivity was distributed throughout the cytoplasm. No adverse animal behavior was noted at any viral dose. Histopathology revealed dose-dependent disseminated areas of necrosis (data not shown) with no differences between Ad-TIMP-2 and Ad-βgal. Taken together, these data demonstrate highly efficient adenoviral gene transfer of TIMP-2 to the liver of nude mice and consequent expression of TIMP-2.

To determine whether TIMP-2 would, as expected, be secreted into the serum in vivo, we measured levels of TIMP-2 3 days after systemic application of increasing doses of Ad-TIMP-2 by ELISA. Whereas the serum levels in uninfected or Ad-βgal-infected controls were below the detection level of 8.0 ng/ml, application of $0.75 \times 10^{10}$ pfu of Ad-TIMP-2, which results in $<10\%$ infected hepatocytes in vivo (data not shown), led to highly elevated serum levels (Fig. 4a). A substantial further increase in systemic TIMP-2 levels was seen at higher doses of Ad-TIMP-2 but not Ad-βgal (Fig. 4a). To evaluate the duration of transgene expression, we monitored TIMP-2 levels over a time of 5 weeks after application of $3 \times 10^{10}$ pfu Ad-TIMP-2 (Fig. 4b). Within the first 14 days, the levels remained stable. After 2 weeks, however, a marked decrease in TIMP-2 expression was observed. Four or 5 weeks after virus application, which was the time when the efficacy experiments were terminated, the levels had decreased 50- or 100-fold but were still clearly above the background (Fig. 4b). In summary, these data demonstrate the efficacy of adenovirus-mediated gene transfer to hepatocytes in vivo for production of secreted diffusible inhibitors.

Prevention of Metastasis by Adenoviral TIMP-2 Gene Transfer to the Liver. In the first set of experiments, $3 \times 10^{10}$ pfu Ad-TIMP-2 or Ad-βgal were administered i.v. Three days later, $2 \times 10^6$ LS174T were injected intrasplenally. Tumor loads in the livers were evaluated after 5 weeks. The average tumor load in Ad-TIMP-treated animals was $<5\%$ of the untreated ($P = 0.002$) or Ad-βgal-treated ($P = 0.017$) controls (Fig. 5, a and b). The majority of the livers of control animals were nearly entirely replaced by tumor (Fig. 5b), and the weights of the tumorous livers were up to three times above normal liver weights. In contrast, in the Ad-TIMP-2 group, three of five animals were macroscopically tumor free, and the other two animals had only small, marginally located metastases (data not shown and Fig. 5c). The histopathological examination revealed only occasional metastases (one to three per slide) in the three animals that were macroscopically tumor free. These results indicate that the development of highly invasive metastases to the liver can be nearly entirely prevented by TIMP-2 overexpression.

Treatment of Well-Established Metastases by Adenoviral TIMP-2 Gene Transfer to the Liver. In a second set of experiments, we examined whether the growth of established metastases could be inhibited by TIMP-2. On day 0, metastases were induced by intra-splenic injection of $2 \times 10^6$ LS174T cells. When multiple metastases of up to 0.7 mm in diameter had formed 10 days later, $3 \times 10^{10}$ or $6 \times 10^{10}$ pfu Ad-TIMP-2 or Ad-βgal were applied systemically. Five weeks after tumor induction, animals were sacrificed, and tumor loads in the liver were determined. Although a small, but not significant, reduction in the average tumor load in the Ad-βgal groups as compared with the nonvirus controls was seen, the reduction in tumor load in the Ad-TIMP-2 groups was significant for both the low-dose ($P = 0.021$) and high-dose ($P = 0.002$) treatments (Fig. 6a). Comparison of treatment groups that received the same viral doses of Ad-βgal and Ad-TIMP-2, respectively, revealed a statistically signif-

Fig. 5. Prevention of liver metastases by systemic application of Ad-TIMP-2. a, tumor masses. Animals that had to be killed before the date are included. Bars, SD. b, representative in situ photographs of untreated (left), Ad-βgal-treated (middle), and Ad-TIMP-2-treated livers (right). c, representative scans of histopathological slides (H&E staining) of an Ad-βgal pretreated (top) and an Ad-TIMP-2 pretreated (bottom) animal. The livers are represented by one representative slide. Parenchymal liver tissue stains lighter (more eosinophilic, long arrows) than tumor tissue (more basophilic, short arrows). Necroses (arrowhead), mainly within tumorous tissue, display a more whitish color.
significant superiority only for low-dose treatment ($P = 0.038$). In the high-dose groups, there was a trend toward smaller volumes.

We next determined whether inhibition of tumor growth would prolong survival of animals and whether the therapeutic outcome was dependent on the initial tumor size. Liver metastases were induced essentially as described above but at the lower dose of $0.5 \times 10^6$ LS174T cells to prolong survival. Ad-TIMP-2 or Ad-βgal as control was then applied after 3, 7, or 10 days to start therapy when tumors are different in size. As shown in Fig. 6b, in all three therapeutic settings, Ad-TIMP-2-treated mice appeared to show an increased survival than Ad-βgal-treated mice. In addition, 4 of 15 animals (27%) in the TIMP-2 groups survived the up-to-now 10-month observation period without disease, whereas all Ad-βgal-treated mice ($n = 15$) died within 4.5 months. These differences were, however, not significant because of the small number of animals in this pilot survival experiment. Also, some animals suffered from involvement of extrahepatic organs, such as the spleen and peritoneum, which may have outbalanced the liver preventive effect of TIMP-2. These combined results indicate that transfer of TIMP-2 can reduce the growth of well-established liver metastases and prolong the survival of treated animals.

Assessment of Proliferative, Angiogenic, and Apoptotic Activity in Metastatic Livers. To further characterize tumors histologically, we assessed proliferative and apoptotic indices of tumors from animals in both preventive and therapeutic experiments. In livers pretreated with adenoviral vectors prior to tumor challenge, Ad-TIMP-2-treated animals had lower proliferative indices than both Ad-βgal-treated and uninfected controls (Fig. 7). This result was reproduced for animals treated after tumor challenge, except for mitoses at high-dose viral infection (Fig. 7). Evaluation of CD31 staining in sections revealed that Ad-TIMP-2 treatment had no significant effect on angiogenesis in either experimental design (Fig. 7). However, these experiments revealed an induction of CD31 staining in high-dose Ad-βgal-treated animals (Fig. 7). Interestingly, TUNEL staining was increased in Ad-TIMP-2-treated mice compared with both Ad-βgal-treated and uninfected animals and in both experimental protocols (Fig. 7).

DISCUSSION

The results of this study show that the formation of colorectal liver metastases and the growth of well-established metastases can be reduced by systemic Ad-TIMP-2 treatment. To our knowledge, this is the first example of successful in vivo gene transfer of an MMP inhibitor for metastasis prevention and treatment. By making use of the natural tropism of adenoviral vectors to liver tissue, we were able to confirm and extend a new concept in cancer gene therapy: the targeting of normal organ tissue with high risk for metastasis by the means of gene therapy. Although the idea of viral infection of otherwise unaffected tissue with all of the imaginable and documented problems of gene therapy may be not appropriate at first glance, the advantages of this approach may prove to be invaluable in the long run. Because multiple metastases require regional or systemic therapy, the accessibility of the target tissue through the bloodstream is of crucial relevance. Because tumorous deposits usually display high intratumoral pressure, are often extensively necrotic and poorly or unevenly vascularized, and in the micrometastatic state not vascularized at all, it is logical to target normal tissue surrounding the tumors, which displays more favorable features for transduction. Recently, the adenoviral transfer of the cytosine deaminase suicide gene to liver tissue reduced the growth of an implanted colorectal tumor in rat livers, and no toxicity was reported (29). Similarly, the adenoviral transfer of plasminogen activator inhibitor type 1, another well-char-
An characterized protease inhibitor, has been shown to reduce metastasis from intraocular melanomas to the liver (30), and the adenoviral transfer of antiangiogenic genes to the liver has resulted in growth inhibition of liver metastases (31, 32). These results underscore the utility of targeting normal tissue, and the combinatorial application of suicide genes and diverse protease inhibitors may provide even higher efficacy than either protein alone.

We were surprised about the extremely high efficacy of metastasis prevention achieved, with three of five animals remaining macroscopically tumor free as compared with extensive metastasis with nearly entire replacement of the liver tissue with tumor in the control groups. Each of the steps of the metastatic cascade after adhesion of the tumor cell to the endothelium may be inhibited by TIMP-2, i.e.: (a) the initial extravasation either from the sinusoids through the Dissé space or from larger portal vessels through the basal membrane; (b) the invasion and degradation of the ECM within the parenchymal tissue; and (c) the inhibition of angiogenesis. The superiority of the preventive approach over the therapeutic approach argues for the importance of TIMP-2 in the inhibition of the earlier stages of metastasis. This is underscored by the fact that preventive expression of TIMP-2 by the host tissue is obviously sufficient for metastasis prevention, whereas the antitumor effect noted in the treatment of established metastases might have been attributable in part to direct adenoviral infection of the tumorous deposits themselves.

Fig. 7. Effect of Ad-TIMP-2 on proliferation, angiogenesis, and apoptosis. Paraffin sections from the livers of mice from both types of efficacy experiments, as described in the legends for Figs. 5 and 6, were stained with H&E to detect mitoses, anti-MIB-1 to detect proliferating cells, anti-CD31 as a marker for angiogenesis, or according to the TUNEL method for detection of apoptotic cells. Data are presented as positive cells per microscopic field or in case of MIB as the percentage of positive cells. The asterisks indicate the level of significance: *, P < 0.05; **, P ≤ 0.01; and ***, P ≤ 0.005. Ad-βgal-treated animals are compared with untreated animals, and Ad-TIMP-2-treated animals are compared with untreated (top asterisk) and Ad-βgal-treated animals that had received the same viral dose (bottom asterisk). Bars, SD.
Available in vitro data demonstrate that adenovirally transferred TIMP-2 and other TIMPs can reduce the invasion of MMP-2-secreting melanoma cells through reconstituted basal membranes (33). It remains to be shown, however, whether this mechanism is the most relevant in vivo. Recently, it was shown that mice transgenic for TIMP-1 and SV40 T antigen developed much smaller liver tumors than controls, although TIMP-1 overexpression could not prevent metastatic challenge (34). Although this effect was interpreted as inhibition of angiogenesis, it argues for a minor importance of the inhibition of extravasation by TIMP-1 and for the diversity of effects that can be expected from different classes of TIMPs. In our model, the efficacy against metastasis formation as well as further growth argues for a participation of several mechanisms in the invasive cascade. Because MMPs are involved in all these steps and the inhibitory effect of TIMP-2 on MMP-2 and other MMPs has been well described, this interaction is probably at least partially responsible for the antimetastatic effects observed. However, we have not presented clear evidence for this assumption, and anti-invasive or other growth-inhibitory effects of TIMP-2, independent from its effects on MMPs, may also be involved. Our extensive histological examination revealed that the observed growth inhibition by Ad-TIMP-2 is paralleled by a statistically significant inhibition of proliferation and a stimulation of apoptosis. These effects could be the indirect result of the inhibition of invasion by TIMP-2, but it is also possible that the effects are exerted by TIMP-2 more directly. Further experimentation is required to elucidate the mechanisms underlying these phenotypic changes. The pronounced histological differences between the treatment groups, 4 or 5 weeks after virus application, were somewhat surprising because serum TIMP-2 levels had already declined 50- or 100-fold by the time the animals were scrutinized for histological examination. One reason for the marked effects on tumor histology, despite low levels of TIMP-2 in the blood, could be that the concentration of TIMP-2 was markedly higher in the liver sinusoids or the hepatic interstitium than in the peripheral blood, and that the local TIMP-2 levels were still sufficient for exerting therapeutic effects at this time. Alternatively, overexpression of TIMP-2 early in tumor development may lead to a severe and long-lasting disturbance of the balance of proliferation and apoptosis, which may be similar to the well-described and long-lasting inhibitory effects of single doses of chemotherapeutic agents.

Several clinical scenarios for our approach are imaginable:

(a) The i.v. injection of Ad-TIMP-2 might serve as a prophylactic adjunct to conventional modalities in cases of nonresectable primary tumors with the risk of metastasization or alternatively before surgery of the primary tumor and during other maneuvers that could lead to blood-borne shedding of cancer cells.

(b) The outgrowth of disseminated micrometastases, which can remain dormant for extended periods of time, may be prevented. This concept will be particularly dependent on the broad availability of vectors with the potential for long-term expression. In this context, it may be advantageous that, other than for the treatment of monogenetic diseases, immunogenicity of the transferred transgene product does not have to be expected, because it is an endogenous gene that becomes overexpressed.

(c) Established metastases could be the target of our approach, although further improvements in treatment efficacy would be desirable to justify a clinical trial.

(d) Other organs besides the liver that are at high risk for metastatic deposits could be targeted if the promising modifications in vector tropism are further extended. A very helpful assessment of the significance of a new approach can be provided by a comparison with more established therapies. In the case of protease inhibitors, clinical trials with synthetic inhibitors of MMPs, such as batimatistat and marimastat, have displayed antitumor efficacy as measured by reduction of the levels of cancer-specific antigens, and some of these substances are already in Phase II/III clinical trials (35). Any success of synthetic inhibitors underscores in principle the utility of systemic inhibition of certain proteases. A gene therapeutic approach could add additional useful features, such as: (a) the achievement of high local concentrations by using the natural tropism of vectors or by modifying the viral surface structures leading to increased efficacy and reduced toxicity; (b) the continuous release of substances over extended periods of time with potentially lower costs for long-term treatment than generated by synthetic inhibitors; and (c) the combination of two or more highly specific protease inhibitors. In this context, it will be of crucial importance to determine which of the mechanisms, the local secretion of TIMP-2 in the target organ or the secretion of TIMP-2 into the blood with the potential for systemic effects, will be of higher relevance or how both principles can be efficiently combined.

In summary, we have described an approach that allows efficient treatment of colorectal liver metastases by targeting and, thereby, protecting normal organ tissue from cancer cell invasion, which may obviate the need for targeting of tumor deposits themselves.

REFERENCES


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