Liver Cancer Protease Activity Profiles Support Therapeutic Options with Matrix Metalloproteinase–Activatable Oncolytic Measles Virus

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Abstract
Primary and secondary cancers of the liver are a significant health problem with limited treatment options. We sought here to develop an oncolytic measles virus (MV) preferentially activated in liver tumor tissue, thus reducing infection and destruction of healthy tissue. We documented that in primary tumor tissue, urokinase-type plasminogen activator and especially matrix metalloproteinase-2 (MMP-2) are significantly more active than in adjacent nontumorous tissue. We then generated variants of the MV fusion protein by inserting different MMP substrate motifs at the protease cleavage site and identified the motif PQGLYA as the most efficient cleavage site as determined by syncytia formation on protease-positive tumor cells. The corresponding MMP-activatable oncolytic MV-MMPA1 virus was rescued and shown to be strongly restricted on primary human hepatocytes and healthy human liver tissue, while remaining as effective as the parental MV in the tumor tissue sections. Our findings underline the clinical potency of the MMP activation concept as a strategy to generate safer oncolytic viruses for the treatment of primary and secondary cancers of the liver.

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Introduction
There is still an unmet need in the treatment of primary and secondary cancers of the liver. Patients with primary hepatocellular carcinoma (HCC) or advanced stage extrahepatic tumors that have metastasized to the liver are usually diagnosed when surgical removal of the tumor has become impossible and chemotherapeutic treatment options are limited. For example, resection of colorectal cancer (CRC) liver metastasis is the most effective approach with a recurrence rate of more than 60% (1) and a 5-year survival rate of less than 50% (2). For patients with unresectable HCC, these numbers are even lower (3). Thus, new treatment modalities for these tumors are urgently needed.

Virotherapy is based on viruses that preferentially infect and destroy tumors. Different species of oncolytic viruses, including measles virus (MV), are currently under clinical development (4, 5), and oncolytic MV has shown antitumor efficacy in preclinical animal models of HCC (6). The selectivity of MV replication and killing of tumor cells has been attributed to a weakened innate cellular immunity and tumor cell–specific overexpression of the MV receptor CD46 (7). Further tumor specificity can be added by engineering the MV glycoproteins [i.e., the hemagglutinin (H), responsible for receptor recognition and attachment, and the fusion protein (F), which mediates fusion of the viral and cellular membranes (8, 9)]. To become fusion active, F has to be processed by the ubiquitously expressed trans-Golgi protease furin. We have previously shown that addition of a matrix metalloprotease (MMP) substrate motif selected from retroviral protease substrate libraries on tumor cells (10, 11) into the F protein prevents fusion activity in the absence of tumor cell–associated proteases, restricts replication of recombinant virus to protease-expressing tumor cells, and strongly attenuates the neurovirulence of MV (12).

MMPs are overexpressed in a wide variety of human tumors and degrade the extracellular matrix surrounding the tumor nodules to facilitate the invasive growth of the tumor mass. Multiple MMP family members, such as collagenase 1 (MMP-1), gelatinase A (MMP-2), gelatinase B (MMP-9), matrilysin (MMP-7), and membrane-type (MT)-MMPs, have been correlated with tumor progression (13). Overexpression of urokinase-type plasminogen activator (uPA) correlates with the formation of metastasis and bad prognosis (14, 15).
In primary HCC and CRC-derived liver metastases, MMP-2 and MMP-9 (CRC metastases) are significantly more active in tumor tissue as compared with healthy liver tissue (16–19). Similarly, the activity of uPA is increased in primary lesions of CRC when compared with neighboring healthy colon tissue (20, 21). However, a systematic analysis of the activities of tumor-associated proteases in tumors and adjacent nontumorous tissues of larger patient cohorts with liver cancer is not yet available.

Here, we analyzed the expression of gelatinases, plasminogen activators, further MMPs, and tissue inhibitor of metalloproteinases (TIMP) in precision-cut liver slice (PCLS) cultures of tumors and nontumorous tissues generated from resection material of a total of 44 human patients suffering from primary and secondary liver cancer. A series of MMP-activatable F proteins was generated and analyzed for MMP activation and fusion activity. The virus based on the most fusion-active F variant was strongly restricted in spreading through primary human hepatocytes and human nontumorous liver tissue.

Materials and Methods

Plasmid construction

Coding sequences for the MMP cleavage site motifs previously selected from retroviral substrate libraries (10) were introduced into the MV-F open reading frame by fusion PCR using pCG-F (22) as template. The plasmid pCG-F-PQ was cloned using the QuikChange site-directed mutagenesis kit (Stratagene) with pCG-F-MMPA1 (12) as template according to the manufacturer’s instructions. Primers and detailed procedures are available on request.

Cells and viruses

Vero (African green monkey kidney, ATCC CCL-81), HT1080 (human fibrosarcoma, ATCC CCL-121), and 293T (human epithelial kidney, ATCC CRL-11268) cells were grown in DMEM (Invitrogen) supplemented with 10% fetal bovine serum (FBS), and 8U/ml human globastoma, ATCC HTB-14) cells in MEM (Invitrogen) with 10% FBS, 2 mmol/L L-Gln, 1 mmol/L sodium pyruvate, 3.35 g/L NaHCO3, and 1% non-essential amino acids. All cell lines were purchased from the American Type Culture Collection (ATCC; authenticity by short tandem repeat profiling, morphology, karyotyping, or isoenzyme analysis) and passaged for fewer than 6 months after receipt or resuscitation. The rescue helper cell line 293-3-46 (23), characterized morphologically and functionally, was grown in DMEM with 10% FBS and 1.2 mg/mL G418. Live primary human hepatocytes were purchased from Lonza (Lonza Verviers SPRL) and cultured in fully supplemented HCM medium (Lonza).

The Edisonston B–based parental MV strain (NSe) encoding green fluorescent protein (GFP) as additional transcription unit and MV-MMPA1 (12) were rescued as described previously (23). The viruses were propagated in Vero or HT1080 cells, respectively. Titers were determined by 50% tissue culture infective dose titration (TCID50) by the method of Kärber (24) on Vero or HT1080 cells.

Human tissues

Human liver resection material from 44 patients suffering from primary and secondary tumors of the liver was obtained by the Department of General, Visceral and Transplant Surgery, University Clinic Tübingen, with informed consent according to the guidelines of the local ethics committee. Explanted tissues were stored immediately in ice-cold William’s E medium (Lonza) and the slicing procedure was started within 1 hour.

Preparation, cultivation, and infection of tissue slices

PCLS were prepared and cultivated as described before (25). Conditioned medium was stored at −80°C before analysis of proteases. Only tissue slices with sufficient viability as assessed by infection with an adenoviral vector encoding GFP (25) were infected the next day after preparation with the respective virus stocks [multiplicity of infection (MOI) of 1] and monitored. Three days after infection, slices and supernatants thereof were harvested and stored at −80°C. Cell extracts were prepared by sonicative in ice-cold radio-immunoprecipitation assay lysis buffer [50 mmol/L Tris (pH 8.0), 62.5 mmol/L EDTA, 1% IGEPAL CA-630 (formerly NP40), 0.4% deoxycholate; Sigma] supplemented with Complete protease inhibitors (Roche Biochemicals).

Zymography

Gelatinase zymography was done as described before (10). Plasminogen zymography was done with the following modifications: The gel contained 0.1% gelatin and 10 μg/mL plasminogen (Calbiochem, Merck KGaA) and overnight proteolysis was performed in 50 mmol/L glycine-NaOH + 10 mmol/L EDTA. For densitometric analysis, stained gels were scanned with a transilluminating scanner (UMAX, Biostep GmbH) and analyzed with the TotalLab analytic software package (Phoretix). Data evaluations were performed using logarithmized ratios of tumorous (T) and nontumorous liver (L) values log2(T/L). Null values were replaced with a value of 100 (lower detection limit) before logarithmizing the data. Comparisons between patient groups and proteases were performed by means of a two-factor ANOVA with the factors patient group and protease and an interaction term. For pairwise comparisons, P values and confidence limits were adjusted with the Bonferroni method to account for multiplicity. The statistical analysis was performed with SAS/STAT software, version 9.2.

Quantification of membrane fusion activity

Cells seeded in 12-well tissue culture plates were transfected with 1 μg each of F and H expression plasmids using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. The MMP inhibitor GM6001 (Calbiochem) was applied at a final concentration of 10 μmol/L. Cells were incubated overnight at 37°C, fixed, stained with crystal violet, and the numbers of nuclei per syncytium were determined.

Immunoblotting

Lysates were clarified by centrifugation at 12,000 × g for 15 minutes at 4°C. Equal amounts of proteins from tissue
slice extracts were denatured for 10 minutes at 95°C in 2x urea sample buffer (5% SDS, 8 mol/L urea, 200 mmol/L Tris-HCl, 0.1 mmol/L EDTA, 0.03% bromphenol blue, 2.5% DTT, pH 8.0), fractionated by SDS-PAGE, and blotted onto polyvinylidene difluoride membranes (GE Healthcare). Membranes were blocked with 5% skim milk powder in TBS + 0.1% Tween 20 for 30 minutes at room temperature. For detection of MN nucleopapsid protein, membranes were incubated with rabbit anti-MV-N (ab23974, Abcam plc) diluted 1:5,000 and peroxidase-conjugated goat anti-rabbit immunoglobulin G (Bio-Rad Laboratories) diluted 1:20,000 as secondary antibody.

**Quantitative reverse transcription-PCR**

Viral RNA was isolated using the QIAamp viral RNA extraction kit (Qiagen) according to the manufacturer’s instructions. Copy numbers of MV-N gene RNA were quantified using the Quantifast SYBR Green reverse transcription-PCR (RT-PCR) kit (Qiagen) with the primers Ns (5’-AGTAGAAGT-GAGCTACC-3’) and Nas (5’-TGCTAGGGGTGTTGCC-3’; ref. 26) on a LightCycler instrument (Roche Diagnostics) according to the manufacturer’s instructions.

**Results**

**Activity profile of tumor-associated proteases in human liver tumors**

Tumor resection material of 44 patients diagnosed with liver cancer was collected. Of these, 11 patients had been diagnosed with HCC, 9 with cholangiocarcinoma (CC), 17 with colorectal carcinoma (CRC) liver metastases, 3 with pancreatic carcinoma liver metastases, and 1 patient each with liver metastases arising from B-cell non-Hodgkin’s lymphoma, uveal melanoma, renal cell carcinoma, or follicular nodular hyperplasia. As described before (25), tumorous, adjacent nontumorous, as well as mixed tumorous/ nontumorous tissue areas were initially defined by an expert pathologist via macroscopic examination and later confirmed by histology. Mixed tissues were excluded from further analysis. PCLS were generated and cultivated for 24 hours before the conditioned supernatant was harvested and analyzed for protease activities.

In gelatinase zymography gels (example shown in Fig. 1A), the activities of both the proform proMMP-9 and the activated form of MMP-9 were detected as a merged band (arrow 3) between the sizes of 92 and 82 kDa. The upper two bands migrating at approximate molecular weights of 220 and 135 kDa (arrows 1 and 2) possibly represent the homodimer of proMMP-9 and the proMMP-9/neutrophil gelatinase–associated lipocalin heterodimer, respectively (27). The two bands at 72 and 62 kDa (Fig. 1A, arrows 4 and 5) represent the proform and the activated form of MMP-2, respectively, as verified by Western blot analysis (data not shown). In plasminogen activator gels (an example is shown in Fig. 1B), we detected weak tissue-type plasminogen activator (tPA) activity (band at 66 kDa, arrow 1) and strong uPA activity (bands at about 50 kDa, arrow 2) in most patients. The relative protease activities of tumorous and nontumorous tissue samples were quantified by densitometric analysis of the zymographies for each patient (Supplementary Table S1). The results revealed that especially the active form of MMP-2 was on average more than 10-fold upregulated in tumorous tissue (Fig. 1C; Supplementary Table S2). There was also an increase in the activities of pro-MMP-2 and uPA, although to a smaller extent (Fig. 1C; Supplementary Table S2). This upregulation of the protease activity in tumor tissue was highly significant. MMP-9 activity, in contrast, did not differ significantly between tumor and nontumor tissues.

When allocated to the specific tumor entities, the overall tendency observed in the whole patient collective was confirmed (Fig. 2). For MMP-9, tumor and liver tissues showed comparable activity (Fig. 2C) with increased activity only for some patients. A tendency for stronger upregulation of protease activity in CC or CRC (e.g., for activated MMP-2, more than an order of magnitude, on average) in comparison with HCC could be observed (Fig. 2A, B, and D; Supplementary Table S3). A statistical analysis comparing tumor and liver tissues displayed a significant upregulation of protease activity for the activated form of MMP-2 and uPA in HCC, CC, and CRC, with the most prominent upregulation observed for the activated form of MMP-2 (Supplementary Table S3).

On a subset of samples, protein array analysis for further types of MMPs and TIMPs was performed. For MMP-1, MMP-3, MMP-10, MMP-13, or TIMP-4, no significant differences between tumorous and nontumorous tissues were detected (data not shown). However, a significant tumor-specific upregulation was observed for MMP-8 and TIMP-2 in 8 and 6 of 14 matched samples, respectively; TIMP-1 was overexpressed in the tumor tissue of 9 of 14 matched pairs (Supplementary Table S4).

**Expanded repertoire of protease cleavage sites for MMP activation of the MV fusion protein**

To expand the repertoire of potential MMP recognition sites, we generated a set of F variants containing MMP recognition sites that had been selected from a retroviral protease substrate library. These sites [single amino acid code sequence: PAGLHV (“HV”) and AKGLYK (“AK”), especially

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of the variants encoding the MMP cleavage site only was detectable in HT1080 cells only (Supplementary Fig. S2).

Interestingly, the AK motif–containing F proteins were processed at wild-type levels, ruling out inefficient cleavage as reason for their defect in membrane fusion (Supplementary Fig. S2). We therefore hypothesized that the COOH-terminal lysine residue in the AK motif, which is supposed to remain next to the NH2-terminal F1 fusion peptide after cleavage, might interfere with the insertion of the fusion peptide into the target cell membrane. To test this hypothesis, we exchanged the crucial lysine residue in variant F-AK against alanine resulting in F-AKA (motif AKGLYA). Moreover, in the wild-type F protein, a single lysine residue was inserted between the furin site and the fusion peptide resulting in variant F-F2KF1 (Fig. 3A). Both variants were properly expressed and processed (Supplementary Fig. S2). Fusion activity was indeed regained for F-AKA, whereas fusion was abolished in the F2KF1 variant (Fig. 3B). This showed the fusion inhibitory activity of positively charged residues at the NH2 terminus of the fusion peptide.

To further show MMP-dependent activation of the different F protein variants, we added the broad-range MMP inhibitor GM6001 to transfected HT1080 cells. Whereas the fusion activity of the unmodified F protein remained unaffected, all other fusion active variants were strongly inhibited (Fig. 3B). Quantification of syncytia formation in the absence of GM6001 showed that the F variants differed substantially in their activities. Whereas F variants with the HV or the AKA motif were moderately active with about 9 to 15 nuclei per syncytium, syncytia induced by the F-PQ and F-MMPA1 variants contained more than 50 nuclei on average (Fig. 3C).

Thus, in context of the MV-F protein, the PQGLYA motif was considerably more effective than the other motifs tested. The corresponding MV-MMPA1 (12) virus was therefore used in the following studies.

**MV-MMPA1 does not spread in normal hepatocytes but selectively destroys MMP-positive tumor cells**

Hepatocytes of three different donors were infected at a low MOI (0.1 or 0.01) with preactivated MV-MMPA1...
(i.e., released from MMP-positive HT1080 cells) or parental MV, both encoding GFP as marker. Whereas strong syncytium formation was observed within 5 days after infection with MV, only a few single hepatocytes became GFP positive on infection with MV-MMPA1. Neither syncytium formation nor virus spread was detected over time for MV-MMPA1 (Fig. 4A). This strong restriction in human hepatocytes was confirmed by Western blot analysis (Fig. 4B) and by quantifying virus RNA in the cell culture supernatant. Whereas signals for MV increased over time, those of MV-MMPA1 remained at a constantly low level and slowly declined (Fig. 4C).

To show the cytolytic activity of MV-MMPA1, we infected Vero cells and MMP-positive HT1080 tumor cells with MV and MV-MMPA1 at low MOI. The number of surviving cells was determined by quantification of cellular colonies appearing 3 days after infection, as previously described (28). As expected, the parental MV exhibited extensive cytotoxicity to Vero cells, whereas MV-MMPA1 was strongly attenuated in this MMP-negative cell line (Fig. 4D). On HT1080 cells, in contrast, both MV and MV-MMPA1 showed a similar cytotoxic effect (Fig. 4D), which is in line with the previously published oncolytic efficacy of MV-MMPA1 in vivo against tumor xenografts (12).

**MV-MMPA1 efficiently infects tumorous but not surrounding nontumorous tissue**

Finally, we followed MV-MMPA1 infection in tissue slices of 10 patients. Three days after infection of the viable tissue samples (nontumorous and tumorous tissues), the parental MV had induced massive syncytia on the nontumorous tissue (Fig. 4A). This strong restriction in human hepatocytes was confirmed by Western blot analysis (Fig. 4B) and by quantifying virus RNA in the cell culture supernatant. Whereas signals for MV increased over time, those of MV-MMPA1 remained at a constantly low level and slowly declined (Fig. 4C).
tissue and showed significant spreading (Fig. 5A, C, and D). Nontumorous slices infected with MV-MMPA1, in contrast, displayed only single infected cells and no spread of infection was observed (Fig. 5A, C, and D). Despite its strong restriction in nontumorous liver tissue, MV-MMPA1 readily infected the tumorous parts of the tissue slices as revealed by microscopy (Fig. 5A, bottom right; Supplementary Fig. S3) and immunoblot analysis (Fig. 5B; Supplementary Fig. S4). The efficient infection of tumor tissue by MV-MMPA1 was quantified by N protein–specific immunoblot analyses (Supplementary Table S5). In six of eight patient samples, we detected more MV-N protein (normalized to β-actin) in MV-MMPA1–infected tumorous tissue than in the corresponding tumor slices infected by MV. The opposite was true for only two patient samples (Fig. 5E). By comparing the amounts of MV-N protein present in tumorous and nontumorous slices, we detected a clear trend ($P = 0.09$, paired Student’s $t$ test) for a preferential infection of tumorous tissue by MV-MMPA1 (Fig. 5F). The extent of tumor targeting determined for the different patient samples correlated considerably with the activities of MMP-2 in the respective tumorous tissues ($r = 0.7388$; Supplementary Fig. S5).

**Discussion**

Preclinical development of oncolytic viruses will be greatly facilitated by model systems reflecting the clinical situation as closely as possible to reliably predict safety and efficacy for first-in-man studies. For viruses with tropism restricted to human and primate cells, such as MV, small-animal models predicting toxic side effects due to infection of nontumorous tissue are difficult to establish. A transgenic mouse expressing the MV receptor CD46 and deficient for the type I IFN receptor allows some virus replication, but systemic propagation is limited and major pathologic effects are restricted to neurotoxicity on intracerebral application (29, 30). We therefore used the PCLS culture technology to assess the spread of oncolytic MV in tissues of patients suffering from primary or secondary cancer of the liver. Moreover, we determined the tumor-specific activity of several proteases in matched slices of adjacent tumorous and nontumorous tissues from a total of 44 patients.

The tissue slice technology allowed us to determine protease activity under native conditions, thereby excluding potential artifacts caused by using tissue lysates (16–18). Moreover, we determined the enzymatic activities of the proteases, an analysis that is more relevant than gene expression studies, as many MMPs undergo complex activation cascades on the protein level (31–34). The differences we observed between the protease activities in tumorous versus nontumorous tissues were remarkable. In almost all of the patients analyzed, especially active MMP-2 and, to a minor extent, uPA were significantly more active, in some patients by more than one order of magnitude, in the tumorous tissue than in the adjacent nontumorous tissue. This was basically the case for all three tumor entities investigated, but being more pronounced in CRC and CC than in HCC patients, which is in line with the role of MMP-2 and uPA as mediators of invasive growth of tumor cells (16–21). However, MMP-9, which also has been implicated in CRC progression, was not found to be differentially active but exhibited high activity levels in both tissue types, indicating that tumor cell
invasion paralleled by liver injury or fibrosis resulted in MMP-9 upregulation in adjacent nontumorous tissue (34–36).

MMP-2 becomes activated at the cell surface, where pro-MMP-2 forms complexes with the membrane-bound activating protease MT1-MMP and the MMP inhibitor TIMP-2. A fine-tuned balance in the amounts of all three proteins seems to be critical for efficient MMP-2 activation (32). We found TIMP-2 to be overexpressed in the tumorous tissue in about half of the patients analyzed (Supplementary Table S4). It is thus likely that upregulation of MMP-2 activity is accompa-

Figure 4. Infection of primary human hepatocytes and cytotoxic activity on cell lines. A, primary human hepatocyte cultures were infected in four replicates at an MOI of 0.1 with MV or MV-MMPA1 or left uninfected (NC). Cultures were inspected daily by fluorescence microscopy for 5 days post-infection (dpi). B, on days 1, 2, 3 and 5, one replica of the infected cultures was lysed and subjected to immunoblot analysis for the MV nucleocapsid (MV-N) protein. C, each day after infection, MV (black) or MV-MMPA1 (gray) released into the supernatant of infected hepatocyte cultures was quantified by quantitative RT-PCR for the copy number of the nucleocapsid (MV-N) protein RNA. D, Vero cells and MMP-positive HT1080 tumor cells were infected with MV or MV-MMPA1 (MOI, 0.1) or left uninfected (NC) in triplicates. Three days after infection, the surviving cells were replated in different dilutions and colony (>50 cells) numbers were counted (number of clones) 11 d after replating.
Figure 5. Infection of human liver tissue slices. Tissue slices of patients S003 (HCC; A and B), S026 (HCC; C), and S028 (CRC; D) were infected with MV or MV-MMPA1 (MOI 1) by calculating a total of $1 \times 10^6$ cells on average for a tissue slice of 8 mm in diameter and 200 to 300 μm in thickness (25). A, fluorescence microscopy of tumorous (T) and nontumorous (L) tissue slices 3 d after infection. Arrow indicates a GFP-positive syncytium in nontumorous tissue induced by infection with unmodified parental MV. B, tumorous (T) and nontumorous (L) tissue slices were infected in duplicates. Three days after infection, the levels of nucleocapsid protein (MV-N) were determined by immunoblot analysis. C, time course of infection in nontumorous tissue slices at 2 and 3 days post-infection (dpi). D, spread of infection on day 3 in nontumorous tissue slices of patient S028. Fluorescence microscopy image of the full area of one representative tissue slice. Typical regions of infectious centers are indicated by the frame (left) and enlarged (right) for each slice. E, pairwise comparison of infectivity of MV and MV-MMPA1 on tumorous tissue slices of patients S002 (CRC), S003 (HCC), S040 (CRC), S041 (FNH), S043 (CC), S044 (CC), S047 (CRC), and S050 (CRC). Relative amounts of viral N protein in infected tumorous tissue slices ($N_t$) were determined 3 d after infection by immunoblot and densitometric analysis with β-actin as standard. F, targeting of MV or MV-MMPA1 to tumorous tissue was analyzed by pairwise evaluation of the ratio of relative viral N protein amounts in tumorous ($N_t$) and nontumorous ($N_n$) tissues of the patient samples listed in E.
substrate motifs of MMP-2 and MT1-MMP (38). Experimental data in this and a previous study showed that both proteases can activate viruses displaying this cleavage site (ref. 11; Supplementary Fig. S6). This illustrates the advantage of library-selected substrate peptides wherein selective advantage is optimal when a substrate peptide becomes cleaved by more than one protease (10).

By introducing further library-selected cleavage sites into the F protein, we made two important observations that will further improve the design and engineering of MMP-activatable MVs. First, charged amino acids are not tolerated in the P1’–P3’ residues that remain NH2-terminal to the hydrophobic fusion peptide after cleavage by MMPs. In particular, a P3’ lysine residue completely abolished membrane fusion activity, which could be restored by exchange with alanine, suggesting that a positively charged residue at this position interferes with the insertion of the fusion peptide into the cellular membrane. Second, the cleavage site for the ubiquitously expressed furin protease, which precedes the MMP cleavage site, is dispensable when library-selected cleavage sites are used, as its presence enhanced neither cleavage efficiency nor membrane fusion activity, as reported for standard MMP cleavage sites (12).

Based on this and a previous study, the MV-MMPA1 virus has now been characterized for a number of preclinically important parameters: (a) MV-MMPA1 replicates selectively in the presence of MMP-2/MT1-MMP and its spreading can be inhibited by MMP-inhibitors (12). (b) It exhibits a strong attenuation of neurotoxicity in a MV mouse model while retaining full oncolytic efficacy on tumor cells and tumor tissue slices (this study) and in tumor xenografts (12). (c) It does not replicate or induce cytopathic effects on cultivated primary human hepatocytes or on nontumorous parts of cultivated liver tissue slices from liver cancer patients. (d) There are no significant differences detectable between MV-MMPA1 and the nontargeted MV on matched tumorous parts of the tissue slices. Although infection studies on patient biopsy material can be influenced by many different parameters, such as differences between individual patients, in pretreatments, or susceptibility to MV infection, the infectivity of MV-MMPA1 correlated with the MMP-2 activities quantified in the biopsy samples. These results show the MMP dependency of MV-MMPA1 accompanied by an uncompromised efficiency in tumor infectivity.

In contrast to this attenuated phenotype of MV-MMPA1, the nontargeted MV spreads readily through primary human hepatocytes and nontumorous liver tissue slices. This poses a risk for liver toxicity when nontargeted MV is applied for the treatment of liver cancer, especially in a scenario of direct loco-regional application of high virus doses. Based on these observations, the absence of liver toxicity on systemic application of MV in the MV mouse model as performed by Blechacz and colleagues (6) should be confirmed by direct intrahepatic applications, possibly using more reliable animal models to exclude liver toxicity before first-in-man studies for the treatment of primary and secondary liver cancer can be initiated. In this regard, patients with strong overactive MMP-2 could be identified and included in first-in-man studies using MMP-activatable MV to suppress liver toxicity. In summary, our data reveal that the MMP activation concept can prevent infection of nonmalignant liver tissue and subsequent liver damage when applying oncolytic MVs for the treatment of solid cancers of the liver.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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