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Differential regulation of matrix metalloproteinases in varicella zoster virus-infected human brain vascular adventitial fibroblasts

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Abstract

Upon reactivation, varicella zoster virus (VZV) spreads transaxonally, infects cerebral arteries and causes ischemic or hemorrhagic stroke, as well as aneurysms. The mechanism(s) of VZV-induced aneurysm formation is unknown. However, matrix metalloproteinases (MMPs), which digest extracellular structural proteins in the artery wall, play a role in cerebral and aortic artery aneurysm formation and rupture. Here, we examined the effect of VZV infection on expression of MMP-1, -2, -3, and -9 in primary human brain vascular adventitial fibroblasts (BRAFS). At 6 days post-infection, VZV- and mock-infected BRAFs were analyzed for mRNA levels of MMP-1, -2, -3 and -9 by RT-PCR and for corresponding total intra- and extracellular protein levels by multiplex ELISA. The activity of MMP-1 was also measured in a substrate cleavage assay. Compared to mock-infected BRAFs, MMP-1, MMP-3 and MMP-9 transcripts, cell lysate protein and conditioned supernatant protein were all increased in VZV-infected BRAFs, whereas MMP-2 transcripts, cell lysate protein and conditioned supernatant protein were decreased. MMP-1 from the conditioned supernatant of VZV-infected BRAFs showed increased cleavage activity on an MMP-1-specific substrate compared to mock-infected BRAFs. Differential regulation of MMPs in VZV-infected BRAFs may contribute to aneurysm formation in VZV vasculopathy.

Keywords

Matrix metalloproteinases; Varicella zoster virus vasculopathy; Aneurysm

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Conflict of interest statement

All authors report no conflicts of interest.

1. Introduction

Primary varicella zoster virus (VZV) infection causes varicella (chickenpox), after which virus becomes latent in ganglionic neurons. In aging and immunocompromised individuals, a decline in cell-mediated immunity to VZV leads to virus reactivation, usually manifesting as herpes zoster (shingles). After reactivation, VZV travels transaxonally to cerebral arteries where pathological vascular remodeling produces VZV vasculopathy possibly leading to aneurysm formation [1,2]. The mechanism by which aneurysms develop in VZV-infected cerebral arteries is unknown. Studies of cerebral and aortic artery aneurysms reveal an association between upregulation of matrix metalloproteinases (MMPs) and aneurysm formation and rupture [3–5]. MMPs are calcium-dependent, zinc-containing endopeptidases that mediate vascular remodeling by degrading extracellular matrix components such as collagen, gelatin and elastin [5]. These endopeptidases are secreted in an inactive form and depend on proteolytic activation as well as interaction with tissue inhibitors of MMPs (TIMPs) for their activity in digesting extracellular structural proteins and disrupting the arterial wall. Herein, we examined MMP expression and activity in VZV- and mock-infected primary human brain vascular adventitial fibroblasts (BRAFs), the arterial cells initially infected during virus reactivation.

2. Materials and methods

Primary human BRAFs (Sciencell, Carlsbad, CA) were obtained from fetal tissue, subcultivated twice more, then seeded at a density of 7,000 cells/cm² in basal fibroblast medium supplemented with 2% fetal bovine serum (FBS), 1% fibroblast growth serum and 1% 100x penicillin-streptomycin (Sciencell). After 24 h, medium was changed to basal fibroblast medium supplemented with 0.1% FBS and 1% 100x penicillin-streptomycin and replenished every 48 h for 7 days to establish quiescence. Quiescent BRAFs were cocultivated with VZV (Ellen strain)-infected or uninfected BRAFs. BRAFs were analyzed at the height of the cytopathic effect (CPE), usually 6 days post-infection (dpi). Messenger RNA was extracted and analyzed by reverse-transcription PCR (RT-PCR) using SYBR green as described [6] and primers for MMP-1, MMP-2, MMP-3, MMP-9 and glyceraldehyde 3-phosphate dehydrogenase (GAPdH) (Fig. 1a). Primer efficiencies were 97%, 97%, 93%, 91% and 102%, respectively. Data were normalized to GAPdH and analyzed using the delta-delta threshold cycle (CT) method [7].

For protein and functional analysis, BRAFs were prepared and infected as described above. At the time of CPE, conditioned supernatant and cell lysates were prepared as described [8] from VZV- and mock-infected BRAFs, and analyzed for levels of MMP-1, -2, -3 and -9 protein by a multiple immunoassay (Meso Scale Discovery, Rockville, MD) according to the manufacturer's directions. Each MMP was quantitated (ng/mL) using a standard curve, and the fold change determined for VZV-infected and mock-infected conditioned supernatant.

To measure MMP-1 functional activity, which represents a balance of MMP activation by proteolysis and the presence of TIMPs, supernatant from VZV- and mock-infected BRAFs at the height of CPE were analyzed in a 96-well format using the colorimetric SensoLyte

Generic MMP Assay Kit per the manufacturer's instructions (AnaSpec, Fremont, CA). Activity levels (ng/mL) of VZV-infected BRAFs were compared to those of mock-infected BRAFs.

3. Results

Compared to mock-infected BRAFs at 6 dpi, VZV-infected BRAFs showed increased levels of MMP-1, MMP-3 and MMP-9 transcripts, cell lysate protein and conditioned supernatant protein, whereas MMP-2 transcripts, cell lysate protein and conditioned supernatant protein were decreased (Fig. 1b, c). Specifically, MMP-1 transcripts were increased 1.53 fold (+0.35; -0.29; $p = 0.17$); MMP-3 transcripts, 3.85 fold (+0.92; -0.74; $p < 0.05$), and MMP-9 transcripts, 3.03 fold (+0.88; -0.68; $p < 0.05$); MMP-2 transcripts were decreased 0.09 fold (+0.00; -0.00; $p < 0.05$). Compared to protein levels in mock-infected supernatants, MMP-1 was increased 5.68 fold (± 0.55 ; $p < 0.05$), MMP-3 protein, 1.69 fold (± 0.16 ; $p = 0.44$), and MMP-9 protein, 2.19 fold (± 0.30 ; $p < 0.05$); MMP-2 protein was decreased 0.82 fold (± 0.005 ; $p = 0.05$) in VZV-infected BRAF supernatants. Compared to protein levels in mock-infected cell lysate, MMP-1 protein was increased 6.03 fold (± 1.01 ; $p = 0.05$), MMP-3 protein, 2.41 fold (± 0.61 ; $p < 0.05$) and MMP-9 protein, 1.77 fold (± 0.37 ; $p = 0.09$); MMP-2 protein was decreased 0.18 fold (± 0.03 ; $p < 0.05$).

MMP-1 from the conditioned supernatant of VZV-infected BRAFs showed increased cleavage activity on an MMP-1-specific substrate [5.53 ng/mL (± 0.70 ; $p < 0.05$)] compared to that from mock-infected BRAFs [0.01 (± 0.02 ; $p < 0.05$) (Fig. 1d).

4. Discussion

Herein, we show that VZV infection of human BRAFs results in altered expression of MMPs, with increased MMP-1, -3 and -9 and decreased MMP-2 mRNA, intracellular protein and extracellular protein. Importantly, MMP-1 cleavage activity of extracellular substrate, which is dependent on proteolytic activation and the activity of TIMPs in the conditioned supernatant, was also increased after VZV infection consistent with previous reports of increased MMP-1 expression in aneurysmal arteries compared to normal cerebral arteries [5,9]. Increased MMP-1 can degrade interstitial collagens and gelatin, leading to focal degradation of the vascular extracellular matrix, aneurysm formation and growth. While increased MMP-3 expression has not been found in intracranial aneurysms, it is increased in macrophage-like mononuclear cells infiltrating the aneurysmal aorta [4]. Finally, MMP-9 has also been shown to be elevated in intracranial aneurysms compared to controls; while perturbations were local rather than systemic, with no significant difference in serum MMP-9 in patients with intracranial aneurysms and controls, more MMP-9 was seen in the aneurysmal wall of intracranial arteries compared to control arteries [10]. MMP-9 is also elevated in the superficial temporal arteries of patients with giant cell arteritis [11], a disease recently linked with VZV infection [12]. Interestingly, while increased MMP-2 has been found in intracranial [13] and abdominal aortic aneurysms [14,15] compared to controls, we found decreased expression of MMP-2 in VZV-infected BRAFs. A similar pattern of decreased MMP-2 and increased MMP-1 and 9 has been described in chronic thoracic aortic dissection [16]. The VZV-induced decrease in MMP-2

may be more related to the role of MMP-2 in cytokine turnover and activation [17], rather than degradation of the cellular matrix.

Finally, our observation that VZV-infected BRAFs have altered MMP expression is consistent with other reports of altered MMP expression after infection with other viruses. For example, HSV-1 infection of human corneal epithelial cells increases MMP-2 expression [18], possibly contributing to accelerated formation of corneal ulcers and necrotic lesions. Epstein-Barr virus-infected nasopharyngeal carcinoma cells have increased MMP-9 which promotes invasion [19]. Respiratory syncytial virus-infected human bronchial epithelial cells stimulates MMP-9 release and knockdown of MMP-9 with siRNA decreases viral titers; concurrent murine studies shows that knockdown of MMP expression also led to decreased lung inflammation and airway resistance [20].

5. Conclusions

Our findings provide a potential mechanism for intracranial aneurysm formation in VZV vasculopathy. Overall, the VZV-induced upregulation of MMP-1, -3 and -9 in brain vascular adventitial fibroblasts is consistent with disruption of vessel wall integrity and aneurysm formation. In addition, MMPs produced by inflammatory cells within virus-infected arteries [21,22] may further potentiate pathological vascular remodeling. Antiviral treatment of a patient with VZV vasculopathy that developed 9 aneurysms resulted in clinical improvement, size reduction of most aneurysms and complete resolution of the 2 largest aneurysms [2]. Further studies are needed to determine the role of individual MMPs in VZV infection and of MMP inhibitors in VZV vasculopathy.

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Abbreviations

VZV	varicella zoster virus
MMP	matrix metalloproteinase
BRAFS	brain vascular adventitial fibroblasts
CPE	cytopathic effect
TIMPs	tissue inhibitors of MMPs
GAPdH	glyceraldehyde 3-phosphate dehydrogenase

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HIGHLIGHTS

- VZV infection of cerebral arteries can cause aneurysm and stroke
- MMP-1, -3 and -9 increased and MMP-2 decreased in VZV-infected adventitial cells
- VZV-induced regulation of MMP expression can contribute to aneurysm formation

Figure 1.

Matrix metalloprotease (MMP)-1, -2, -3 and -9 expression in VZV-infected brain vascular adventitial fibroblasts (BRAFs) and mock-infected controls. mRNA was extracted from VZV- and mock-infected quiescent BRAFs and analyzed by RT-PCR 6 days post-infection (dpi) using primers for MMP-1, -2, -3 -9 and GAPdH (a); VZV transcripts were normalized to GAPdH, followed by delta-delta *CT* analysis. Compared to levels in mock-infected cells, VZV-infected BRAF MMP-1 transcripts were increased 1.53 fold (+0.35; -0.29; $p = 0.17$); MMP-3 transcripts, 3.85 fold (+0.92; -0.74; $p < 0.05$), and MMP-9 transcripts, 3.03 fold (+0.88; -0.68; $p < 0.05$); MMP-2 transcripts were decreased 0.09 fold (+0.00; -0.00; $p < 0.05$); solid line represents no change in transcript levels from VZV-infected and mock-infected BRAFs (b). Conditioned supernatant and cell lysates from VZV- and mock-infected BRAFs at 6 dpi were analyzed for levels of MMP-1, -2, -3 and -9 protein using a multiplex ELISA assay. Compared to levels in mock-infected supernatants, MMP-1 protein was increased 5.68-fold (± 0.55 ; $p < 0.05$), MMP-3 protein, 1.69 fold (± 0.16 ; $p = 0.44$), and MMP-9 protein, 2.19 fold (± 0.30 ; $p < 0.05$); MMP-2 protein was decreased 0.82 fold (± 0.005 ; $p = 0.05$) in VZV-infected BRAF supernatants. Compared to mock-infected cell lysate, MMP-1 protein was increased 6.03 fold (± 1.01 ; $p = 0.05$), MMP-3 protein was

increased 2.41 fold (± 0.61 ; $p < 0.05$) and MMP-9 protein was increased 1.77 fold (± 0.37 ; $p = 0.09$); MMP-2 protein was decreased 0.18 fold (± 0.03 ; $p < 0.05$) (*c*). When MMP-1, which had the greatest fold increase in secreted total protein, was further analyzed for its ability to cleave MMP-1 substrate, cleavage activity in the supernatant from VZV-infected BRAFs was 5.53 ng/mL (± 0.70 ; $p < 0.05$) compared to 0.01 (± 0.02 ; $p < 0.05$) from mock-infected BRAFs (*d*). Data are from 4 independent experiments.