MMP-9 and uPAR regulated glioma cell migration

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Abstract

Integrin dependent and independent MMP-9 and uPAR signaling plays a key role in glioma cell migration and invasion. In this article, we comment on all the possible pathways and molecules associated with MMP-9- and uPAR- mediated glioma cell migration with a special emphasis on integrins, a family of cell adhesion molecules. Our recent research investigations highlighted the substantial benefit of silencing both MMP-9 and uPAR together compared to their individual treatments in glioma. Simultaneous knockdown of both MMP-9 and uPAR regulated a majority of the molecules associated with glioma cell migration and significantly reduced the migration potential of glioma cells. Our results point out that the bicistronic construct, which can simultaneously silence both MMP-9 and uPAR offers a great therapeutic potential and is worth developing as a new drug for treating GBM patients.

Cancer cell migration and invasion are initial steps in metastasis, which is a primary cause of cancer-related death. Strategies to treat infiltrating gliomas, such as chemotherapy and gene therapy, have remained largely unsuccessful and the property that makes glioma resistant to treatment is the tendency of the tumor cells to invade normal brain tissue.[1] Approximately 60% of all primary brain tumors in adults are malignant gliomas (anaplastic astrocytoma, anaplastic oligodendroglioma and glioblastoma multiforme). Glioblastoma multiforme (GBM) is the most common and highly aggressive malignant neoplasm of the central nervous system. GBM cells secrete matrix metalloproteinases (MMPs). A significant correlation between MMP-9 levels and the histological grade of malignancy has already been reported.[2-5] Our recent studies clearly demonstrated the role of MMP-9 and the associated molecular mechanisms in cancer cell migration.[6-9]

In the context of cell motility, the extracellular matrix (ECM) is both a requirement and a physical barrier for cell movement. The ECM provides physical support and organization to tissues. It is a complex assembly of proteins and polysaccharides that are secreted, assembled and modeled by cells. A well-defined brain ECM exists in the form of a true basement membrane, cerebral vasculature and the glial limitans externa. The cerebral vascular basement membrane, which surrounds the blood vessels of the brain, contains type-IV and type-V collagens, laminin, fibronectin, and heparan-sulphate proteoglycans.[10] Type IV collagen and laminin, which are mainly present in the capillaries and large blood vessels, are the main constituents of most basement membranes. Laminin describes a large group of adhesion glycoproteins that are found in all basement membranes and in hyperplastic blood vessels in gliomas,

gliosarcomas and meningiomas, as an integral part of the glial limitans externa. Fibronectin is found at the gliomesenchymal junction of tumors and in tumor-associated blood vessels. Advanced stages of glioblastoma have been shown to express vitronectin, a component of the ECM that is usually absent from normal brain and early-stage gliomas. Tenascin-C, another ECM proteoglycan, is synthesized by glial and neural-crest cells, as well as by satellite cells of the peripheral nervous system. Cells express plasma membrane receptors such as integrins, a family of cell adhesion molecules that bind to ECM components. Cell migration therefore often involves the coordination of ECM proteolysis, adhesion and signaling. Integrins are involved in interactions between the cell and the surrounding ECM and play a central role in cell migration. Integrins expressed in tumor cells contribute to tumor progression and metastasis by increasing tumor cell migration, invasion, proliferation and survival.[11] Interactions between integrins expressed by glioma cells and the ECM and the activity of MMPs form the basis for glioma cell migration and invasion.[12]

Similar to MMP-9, the expression of urokinase-type plasminogen activator receptor (uPAR) is much more robust in high-grade than in low-grade human gliomas.[13] Localization of uPAR mRNA in astrocytoma cells and the endothelial cells within brain tumor tissue has been reported.[13] uPAR regulates proteolysis by binding the extracellular protease uPA and also activates many intracellular signaling pathways.[14] Coordination of uPAR with ECM proteolysis and cell signaling underlies its important function in cell migration, proliferation and survival. The most important transmembrane receptors associated with uPAR signaling are the integrin family of ECM receptors. Integrins are essential uPAR signaling co-receptors and the interactions between uPAR-β1 and uPAR-

β3 have an important role in signaling for cell migration and invasion.[14] uPAR localizes to integrin-containing adhesion complexes and co-immunoprecipitates with integrins and integrin-associated signaling molecules such as FAK and Src family kinases.[15-21] uPAR-β1 integrin interactions are associated with the activation of FAK and ERK, whereas uPAR-β3 integrin interactions are associated with the activation of Rac.[14] uPAR-β1 integrin signaling to ERK and Src increases the expression of uPA and MMPs through AP1 transcription factors.[22-24] Although the activation of ERK by uPAR has been considered primarily to promote cell proliferation or protease expression, myosin light chain kinase (MLCK), the cytoplasmic ERK target can regulate cell motility. uPAR signaling activates MLCK and contributes to induction of cell motility in human tumor cells.[25] uPAR-β3 integrin interaction has an important role in signaling for cell migration through activation of the Rho family small GTPase Rac. Blockade of the uPAR function in tumor cell lines inhibits Rac activation and cell motility, whereas ectopic expression of uPAR drives Rac activation.[14] uPAR and integrin-driven activation of Rac allows assembly of filamentous actin (F-actin)-containing membrane protrusions that extend the leading edge of the cell forward, while pericellular proteolysis removes ECM barriers that would impede the extension of these protrusions.

Upregulation of α2β1, α3β1, α5β1, αVβ3 and α6β1 integrins on GBM have already been reported.[26,27] Collagens, fibronectin, laminin, vitronectin, invasin, osteopontin, prothrombin and thrombospondin serve as extracellular ligands for these integrins.[28] It was recently found that α9β1 integrin played a significant role in the progression of glioblastoma.[29] Integrin α9β1 is classified within a two member sub-family of integrins highlighted in part by its specialized role in cell migration.[30] Tenascin

is a ligand for α9β1 integrin. α9β1 has distinguished its functionality from other integrins by facilitating accelerated cell migration.[31] Unlike other integrin heterodimers, α9β1 was able to both increase cell migration and inhibit cell spreading.[32,33] Our recent studies clearly demonstrated the role played by α9β1 integrin in glioma cell migration.[6,8] α9β1 ligation can activate signaling through Src and FAK- mediated tyrosine phosphorylation of multiple proteins including p130Cas and paxillin.[33,34] Unlike other integrins, α9β1 has been proposed to utilize inducible nitric oxide synthase (iNOS)-nitric oxide (NO) and spermidine/spermine acetyl transferase (SSAT)-inward rectifier potassium channel (Kir) pathways along with common integrin signaling proteins such as Src and FAK to transduce cell migration.[30]

Recently, we have reported the physical interactions that exist among uPAR, MMP-9, β1 integrin, α9 integrin and SSAT in the context of glioma cell migration.[8,35] Cooperation between MMP-9 and integrins is known to activate αVβ3, which strongly enhanced tumor migration[36]. Further, uPAR knockdown in glioma cells reduced the expression of αVβ3 and associated glioma cell migration.[37] Although the interaction of uPAR with integrins is well reported by several investigators, the physical association of MMP-9 with these molecules remains unclear. In addition to mediating glioma cell migration via integrins, MMP-9 acts as a processing enzyme for CD44 cleavage.[9] CD44 is a single chain, transmembrane glycoprotein that is widely expressed in physiological and pathological conditions. CD44 is implicated in cell-cell and cell-matrix adhesion, migration and signaling. CD44 expression is prominent in GBM tissue samples.[9] In addition, we noticed a strong physical interaction between MMP-9 and CD44. Direct interaction of MMP-9 with CD44 promotes cleavage of the later into extracellular and

intracellular domains that are involved in glioma cell migration and adhesion, respectively. It was suggested that the cleaved extracellular domain of CD44 induces cell crawling at the leading edge on a hyaluronic acid matrix, along with lamellipod extension which induces mechanical stretching of cells, triggering extracellular calcium ion flux through stretch-activated calcium channels [38]. MMP-9 knockdown in these glioma cells inhibited MMP-9-mediated proteolytic cleavage of CD44. Therefore, the reduced glioma cell migration after MMP-9 knockdown could also attribute to the inhibition of CD44 cleavage. In this scenario, it appears that the simultaneous knockdown of both MMP-9 and uPAR offers a substantial reduction in cancer cell migration compared to their individual knockdowns.

In the recent past, we reported that the transcriptional inactivation of both MMP-9 and uPAR in combination by shRNA-mediated gene silencing offered a prominent and significant reduction in glioma cell migration and invasion.[6] The reduced glioma cell migration could be attributed to the regulation of several pathways and molecules associated with cell migration, which are downstream to both MMP-9 and uPAR. MMP-9 and uPAR knockdown in glioma cells reduced FAK, Src, and F-actin expressions.[6] In addition, the combined inhibition of MMP-9 and uPAR reduced SSAT expression in glioma cells that results in the elevated intracellular levels of spermidine and spermine with subsequent blockade of Kir 4.2 potassium channel.[8] Although the study revealed the involvement of SSAT-potassium channel pathway in glioma cell migration mediated by MMP-9 and uPAR, we failed to show that it is directly mediated via $\alpha 9\beta 1$ integrin. However, in our earlier study, we could show that the blockade of $\alpha 9\beta 1$ integrin significantly inhibited the increased migration potential of glioma cells in MMP-9/uPAR

overexpressed cells. Although part of the inhibition in migration potential after α9β1 integrin blockade is attributed to SSAT-potassium channel pathway involvement, we cannot rule out the possibility of α9β1-iNOS pathway involvement in MMP-9-/uPAR-mediated glioma cell migration. Our future studies will elucidate the significance of α9β1-iNOS pathway involvement in MMP-9-/uPAR-mediated cancer cell migration. In addition to the regulation of cancer cell migration and invasion, combined inhibition of MMP-9 and uPAR by gene silencing technology reduced glioma cell proliferation, tumor growth and angiogenesis and induced apoptosis.[6,8,39-42] Taken together, simultaneous inhibition of MMP-9 and uPAR by plasmid shRNA construct (pMU or MU-sh) significantly inhibited cancer cell migration by controlling all the possible mechanisms, and this construct appears to have a great therapeutic potential to develop as a new drug for treating GBM patients.

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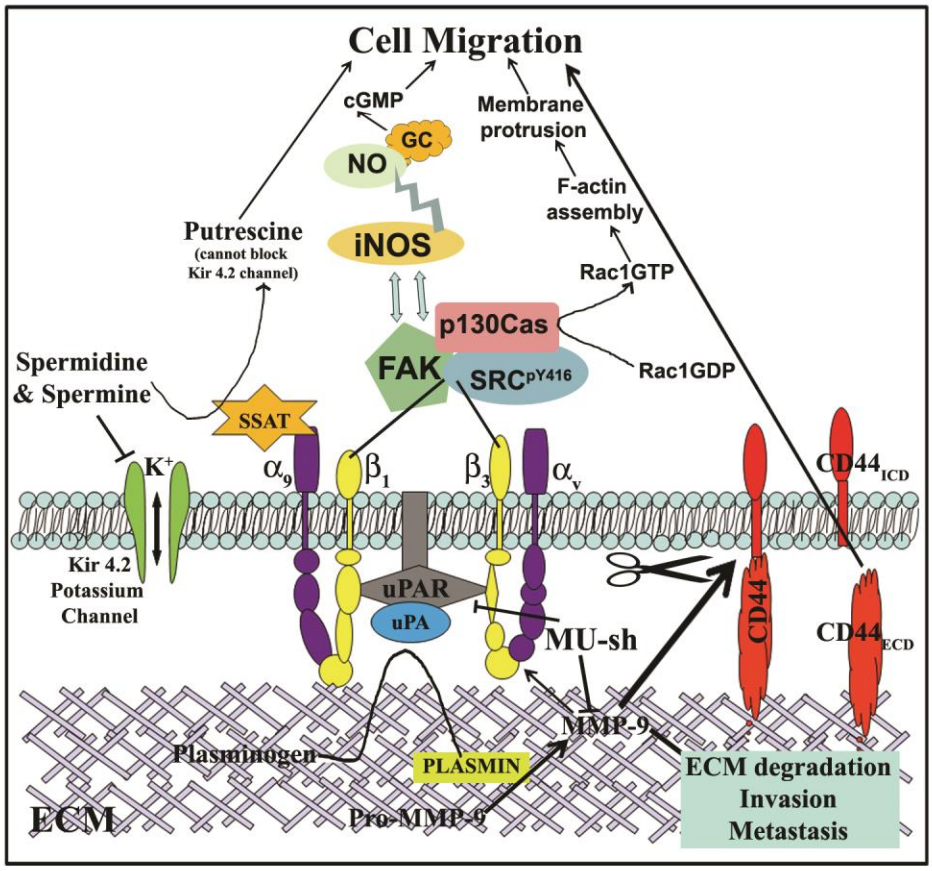

FIGURE LEGEND

Figure 1: Schematic presentation of the possible role and the mechanisms by which MMP-9/uPAR plasmid shRNA (MU-sh) regulate glioma cell migration. ECM-extracellular matrix; ECD-extracellular domain; ICD-intracellular domain; SSAT-spermidine/spermine-N^1-acetyl transferase; GC-guanylyl cyclase; NO-nitric oxide; NOS-nitric oxide synthase.