Gliomas are the most common primary intracranial tumors. Their distinct ability to infiltrate into the extracellular matrix (ECM) of the brain makes it impossible to treat these tumors using surgery and radiation therapy. A number of different studies have suggested that hyaluronan (HA), the principal glycosaminoglycan (GAG) in the ECM of the brain, is the critical factor for glioma invasion. HA-induced glioma invasion was driven by two important molecular events: matrix metalloproteinase (MMP) secretion and upregulation of cell migration. MMP secretion was triggered by HA-induced focal adhesion kinase (FAK) activation, which transmits its signal through ERK activation and nuclear factor kappa B (NFκB) translocation. Another important molecular event is osteopontin (OPN) expression. OPN expression by AKT activation triggers cell migration. These results suggest that HA-induced glioma invasion is tightly regulated by signaling mechanisms, and a detailed understanding of this molecular mechanism will provide important clues for glioma treatment.

Malignant gliomas are highly invasive and infiltrative tumors that have a poor prognosis with a median survival of only one year. A major barrier to effective malignant glioma treatment is the invasion of these cells into brain parenchyma. Because of this fact, local therapies such as surgery or radiation therapy are not effective. Glioma cells invade through the ECM of the brain by activating a number of coordinated cellular programs, which include those necessary for migration and invasion. Therefore, a detailed understanding of the mechanisms underlying this invasive behavior is essential for the development of novel effective therapies.

During glioma invasion, tumor cells closely interact with the ECM. Although brain tumor cells may share some of the invasive characteristics with tumors that arise outside of the central nervous system (CNS), the particular structure and composition of the brain ECM suggest the existence of unique invasive mechanisms for brain tumors. Brain ECM is composed of typical ECM proteins and a HA scaffold with associated glycoproteins and proteoglycans. Typical ECM proteins such as laminin, type-IV collagen and fibronectin have been implicated in the invasion of other tumors by regulating cell adhesion and migration. However HA, which is associated with proteoglycans and GAGs, is especially abundant in the brain parenchyma compared to other tissues. Furthermore, malignant gliomas contain higher amounts of HA than normal brain tissue. These facts raise the possibility that HA might play an important role in glioma invasion, a process that is distinct from other non-CNS derived tumors.

**HA Structure and Function**

HA is a simple but unusual polysaccharide. It is a member of GAG family and is synthesized as a large, negatively charged, unbranched polymer that is composed of repeating disaccharides of glucuronic acid and N-acetylgalactosamine. It has a simple chemical structure but differs in many ways from other GAGs. First, there are 10,000 or more disaccharide repeating units in HA, making it 10^5–10^6 kDa in molecular weight. Second, unlike other GAGs, which include heparan sulphate and chondroitin sulphate, HA contains no sulfate groups or epimerized uronic acid residues. Third, HA is synthesized at the inner face of the plasma membrane as a free linear polymer without any protein core, while other GAGs are synthesized by resident Golgi enzymes and covalently attached to core proteins.

As an ECM component, HA plays a dual function. In normal ECM, HA provides tissue homeostasis, biomechanical integrity, and structure and assembly of tissues. However in malignant tumor tissues, HA transmit signals into cytoplasm and increases tumor cell proliferation, motility and invasion. However, the molecular mechanism of HA-induced tumor progression is divergent and tissue specific.

**Expression of HA in Cancer**

HA contributes to certain types of cancer development. In addition to extracellular HA, intracellular and nuclear forms of HA have been detected. Intracellular HA is involved in cell signaling, whereas nuclear HA could promote chromatin condensation and thus facilitate mitosis. HA expression is frequently increased in malignant tumors. Histological studies demonstrate that HA concentrations are usually higher in malignant tumors than in corresponding benign or normal tissues. HA levels can be increased around tumor cells themselves or within the tumor stroma. Boregowda et al. reported that highly differentiated tumors such as lung, breast, colon, kidney, prostate, astrocytomas and non-Hodgkin’s lymphoma expressed increased amounts of HA in both tumor epithelia and the intratumoral areas.
HA and Prognosis

The level of HA in tumor cells is predictive of malignancy and often correlates with cancer aggressiveness in patients with breast cancers, ovarian carcinomas, non-small-cell lung cancers and prostate cancer. For instance, a high proportion of HA-positive cancer cells and a high intensity of the HA-signal predicts a poor survival rate in colon carcinomas. In addition, Auvinen et al. reported that both the intensity of stromal HA signal alone and that combined with the HA positivity in tumor cells were independent prognostic factors for overall survival in breast carcinoma patients.

HAS in Cancer

HA is made by HA synthases (HAS1, HAS2 and HAS3). Dysregulation of HAS genes results in abnormal production of HA and promotion of abnormal biological processes such as transformation and metastasis. It is thought that HA facilitates tumor growth by opening up spaces for the tumor to migrate to and by interacting with HA binding molecules, assisting in tumor cell adhesion and migration. HA-induced tissue hydration physically creates spaces through which tumor cells may migrate and invade. HA-rich matrices within the tumor-associated stroma are also infiltrated by newly forming blood vessels. A number of cancers are associated with elevated expression of HAS. In animal models, the overexpression of HAS promotes growth of fibrosarcoma and prostate carcinoma, as well as metastasis of mammary carcinoma.

HA as a Cancer Marker

HA levels are often increased in the sera of patients with a variety of different tumors. It has also been shown that HA is significantly elevated in the sera of patients with metastatic disease compared to the sera of patients without metastatic disease. In addition, urinary HA and HAase levels correlate with levels that can be detected in tissues. Likewise, elevated levels of HA and HAase in the urine form a clinically reliable marker for the presence and grade of bladder cancer and prostate cancer. Therefore, HA is of considerable interest as a tumor marker in different cancers.

HA Receptors and Intracellular Signaling

Newly synthesized HA may be secreted and subsequently interact with several cell surface receptors such as cluster determinant 44 (CD44), receptor for hyaluronate-mediated motility (RHAMM), lymphatic vessel endothelial HA receptor (LYVE-1), hyaluronan receptor for endocytosis (HARE), liver endothelial cell clearance receptor and Toll-like receptor-4. Of these, CD44 and RHAMM are well known as signal-transducing receptors that influence cell proliferation, survival and motility. Furthermore, they are known to be closely related to tumor progression.

CD44

CD44, considered the principal receptor for HA, is a multifunctional single-pass transmembrane glycoprotein consisting of four functional domains: an amino terminal domain, stem structure, transmembrane domain and cytoplasmic domain. The amino terminal domain contains motifs that provide docking sites for the ECM components such as HA and other GAGs. The stem structure domain links the amino-terminal domain and transmembrane domain. Stem structure is enlarged by alternative splicing, particularly during tissue repair or in cancer cells. The cytoplasmic domain of CD44 binds to c-Src kinase at a single tyrosine kinase activity that leads to increased tumor cell growth. CD44 and RHAMM are especially important for tumorigenesis and tumor progression by activating downstream signaling molecules, but how this receptor transmits signal to downstream targets is still unclear.

RHAMM

RHAMM is also well known as a HA-binding protein. RHAMM is expressed on the cell surface and in the cytoplasm, as well as in the cytoskeleton and nucleus. Like CD44, RHAMM is subject to alternative splicing, particularly during tissue repair or in cancer cells. Interactions of HA with RHAMM can trigger a number of cellular signaling pathways including those that involve protein kinase C, FAK, NAP kinases, NFκB, RAS, phosphatidylinositol kinase (PI3K), tyrosine kinases and cytoskeletal components. Although it is clear that CD44 and RHAMM can participate independently in proliferative and migratory phenomena, their relative contributions to any given event have not been fully resolved in most cases, and it is likely that they have redundant or overlapping functions in some situations. In general, the interactions of HA with CD44 and RHAMM are especially important for tumorigenesis and tumor progression by activating downstream signaling molecules, but how this receptor transmits signal to downstream targets is still unclear.

Coupling with Other Receptors

CD44 is tightly coupled with receptor tyrosine kinases, p185HER2 and non-receptor tyrosine kinases, c-Src family kinases. CD44 and p185HER2 are physically linked to each other via interchain disulfide bonds and HA can stimulate CD44-associated p185HER2 tyrosine kinase activity that leads to increased tumor cell growth. The cytoplasmic domain of CD44 binds to c-Src kinase at a single site with high affinity. Importantly, HA interaction with CD44 stimulates c-Src kinase activity, increasing tyrosine phosphorylation of cytoskeletal proteins such as cortactin. The binding of HA to CD44 isoforms, which complex with p185HER2 and c-Src kinase, likely triggers direct coupling with two tyrosine kinase-linked signaling pathways during tumor progression. This pathway can explain partly how HA receptors activate downstream targets to promote tumor progression.

Lipid Rafts

Although a number of binding partners of CD44 have been reported, the mechanisms of signal transduction via CD44 remain
sylphosphatidyl inositol (GPI) anchored glycoproteins, which are
and lipid rafts because this behavior is similar to that of most glyco-
provide an important link between the CD44 signaling pathway
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poorly understood. One of the most important signaling events
following stimulation via CD44 is tyrosine phosphorylation of intra-
cellular protein substrates. The Src-family non-receptor PTKs such
as Lck, Fyn, Lyn and Hck were shown to be coupled to CD44.62-65
Moreover, CD44 resists solubilization in nonionic detergents, and
recent investigations have shown that CD44 molecules lacking the
cytoplasmic tail are still detergent insoluble, possibly because of their
association with Triton X-100 insoluble lipids.66,67 These results
provide an important link between the CD44 signaling pathway and
lipid rafts because this behavior is similar to that of most glyco-
sphosphatidyl inositol (GPI) anchored glycoproteins, which are
mostly confined to lipid rafts.68 Co-isolation of CD44 with micro-
domains strongly suggests that CD44 generates cellular activation
signals utilizing the signaling machinery of the lipid rafts.

HA-induced MMP Secretion in Glioma Invasion

Glioblastoma is a severe type of primary brain tumor and irrevers-
ibly infiltrate the normal CNS by the interaction with ECM. One of
the major component in brain ECM is HA, and glioma cells express
high levels of the HA receptors, CD44 and RHAMM.7,69,70 Both of
these HA receptors were known to play an important role for glioma
migration and invasion.71,72

Another important player of glioma invasion is of MMPs. Several
studies have demonstrated the increased expression of MMPs in
gliomas, and GBM and anaplastic astrocytomas. They express higher
levels of MMP-2 and -9 than do low-grade astrocytomas.73 However,
the relationship between HA signaling and MMP secretion was not
well understood.

PTEN is one of the most frequently mutated tumor suppressors in GBM. PTEN also is known to suppresses glioma invasion by
regulating MMP secretion.74 Moreover, we reported that PTEN
suppresses HA-induced secretion of MMP-9 and inhibits HA-induced
invasion in U87MG cells, probably via dephosphorylation of FAK
(see Fig. 1).75 We further addressed that FAK is direct substrate of
PTEN as a protein phosphatase.74 These results suggest that PTEN
mutation is inevitable for the HA-induced glioma invasion by regu-
lar MMP-9 secretion.

FAK, one of main substrates of the PTEN protein, is upregu-
lated in GBMs, particularly in invasive zones. FAK overexpression
has been correlated with the invasive potential of a tumor and poor
patient prognosis.75,76 In HA-stimulated glioma invasion, FAK is
required for the Ras-ERK 1/2 signaling pathway and modulates
MMP-9 secretion (Fig. 1).77,78

Another important signaling event which regulates MMP expres-
sion is the transcriptional activation of genes whose promoter
sequences contain putative binding sites for AP-1 and/or NFKB.79
NFKB is in a family of transcription factors that have been shown
to be involved in regulation of cellular processes like inflam-
mation, immune response, cell proliferation and apoptosis.80 NFKB
activity is regulated by the endogenous inhibitor IκBα; interaction
of NFKB with IκBα blocks the nuclear transport signal and keeps
it sequestered in the cytoplasm. Following any kind of stimulation,
IκBα is phosphorylated at serine residues 32 and 36, which leads to
its ubiquitination and degradation. The free NFKB then translocates
to nucleus and activates the transcription of target genes.81 A number
of upstream kinases such as NFKB-inducing kinase (NIK), PI3K and
MEKK play significant roles in regulation of activation of IκK. In
a recent study, we have demonstrated that HA elevates the levels of
MMP-9 mRNA through NFKB activation (Fig. 1).82 We further
showed that HA-induced NFKB activation through phosphorylation
and degradation of IκBα by activating IκK was mediated by FAK
phosphorylation (Fig. 1). These results suggest that regulation of
FAK is the most important step in blocking HA-induced MMP-9
secretion.

HA-Induced OPN Expression in Glioma Migration

As discussed above, glioma invasion is enhanced not only by MMP
secretion but also by upregulation of motility. Several studies have
suggested that HA plays an important role in glioma cell motility
and invasion, but the molecular mechanisms of HA-associated
motility were not well understood.74,82 Recently, we identified group
of genes which can be differentially regulated by HA treatment and
demonstrated that OPN, one of the transcriptional targets of HA, is
responsible for the stimulation of glioma motility by HA treatment
(Fig. 1).83 OPN, a highly phosphorylated glycoprotein of the ECM,
is known to promote cell attachment, spreading and motility, as well
as more complex events like vascular remodeling, bone mineraliza-
tion and tumor metastasis by interacting with its cell surface receptors
CD44 and the αv-containing integrins.84,85 Interestingly, many of the
basic cellular functions affected by OPN are also controlled by HA.
Moreover, OPN and HA are frequently overexpressed in a variety
of malignant cells and the expression levels of OPN and HA have
been correlated with degrees of glioma malignancy.86 These results suggest that OPN induction might be involved in the stimulation of HA-induced cell motility.

PTEN also suppresses migration, as genetic deletion of the PTEN tumor suppressor gene promotes cell motility and PTEN reconstitution or overexpression inhibits cell motility in a variety of cell type.87,88 The signaling mechanism that upregulates OPN expression was also under the control of PTEN activity.85 OPN expression was upregulated by the PI3K/AKT/mTOR pathway in U87MG cells after treatment by HA in PTEN deleted glioma cells and reconstitution of PTEN suppressed OPN expression (Fig 1).83 These results suggest that PTEN suppresses glioma motility and invasion by the inhibition of HA-induced OPN expression and MMP-9 secretion.

Emodin as an Anti-Cancer Drug Against Glioma Invasion

Since HA is a primary ECM component that can stimulate glioma invasion, it makes inhibitors of this signaling as attractive candidates for therapeutic agents. Among protein tyrosine kinase inhibitors, we found that emodin (3-methyl-1,6,8-trihydroxyanthraquinone) has strong anti-invasive activity for HA-induced glioma invasion (Fig. 1).82 Emodin is one of the main active components contained in the root and rhizome of Rheum palmatum L. Emodin has been shown to have a number of biological activities, including antiviral, antimicrobial, immunosuppressive, hepatoprotective, anti-inflammatory and anticancer effects.89-91 Especially in glioma cells, emodin was known to suppress the TGFbeta and FGF-2 induced expression of syndecan-1, a major cell surface heparin sulfate proteolycan, which was specific to malignant gliomas and was absent in normal brain tissues and it may participate in the motility of glioma cells.92 Emodin suppressed the expression of MMP-2 and MMP-9 at both transcriptional and translational levels.82 Pharmacological studies indicated that emodin significantly decreases the activation of FAK, AKT and ERK1/2, thereby suppressing MMP production (Fig. 1).82 We further observed that emodin efficiently suppressed the HA-stimulated AP-1 and NFkB promoter activities.82 In an in vivo tumor progression model, the activity of Mx1 gene was suppressed by emodin treatment.82 These results indicate that oral administration of emodin effectively suppresses Mx1 gene expression in human glioma through inhibition of AKT and ERK1/2 activation in vivo. These findings suggest that emodin may be a clinically valuable anti-cancer agent against gliomas by blocking FAK and AKT activation, which is the key signaling event of HA-induced MMP secretion and cell motility.

Conclusions and Future Directions

Understanding of molecular mechanisms of glioma invasion is critical in developing novel therapeutic strategies or treatments because major therapeutic approaches such as surgery and radiotherapy are useless without blocking glioma invasion. In this review, we have demonstrated that HA is a major player in the brain ECM that cooperates with PTEN-deletion during glioma invasion. These results provide strong evidence for the emerging concept in cancer research that the microenvironment modulates the effects of genetic alterations. PTEN is a tumor suppressor that plays important roles by blocking HA-induced FAK activation and AKT activation. FAK and AKT activation is important for the secretion of MMP and motility of cells. By using emodin, a protein tyrosine kinase inhibitor, we have demonstrated that blocking HA signaling partially reduced tumor size. These results suggest that chemotherapeutic treatments that can effectively block HA induced signaling might be clinically valuable as an anti-invasive agent for gliomas.

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