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REVIEW PAPER

The Pivotal Novel Pathogenic Roles of Hyaluronic Acid and its Receptors in Gliomas

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ABSTRACT

Hyaluronan (hyaluronic acid = HA) is ubiquitously present in the extracellular matrix (ECM) of several mammalian tissues. HA is predominantly well secreted by the white matter of the central nervous system (CNS). Also, HA is much more abundant in the ECM of gliomas than in normal brain. CD44 and Receptor for hyaluronate-mediated motility (RHAMM) are both receptors for HA. CD44 interrelation with HA triggers cell migration while RHAMM interrelation with HA stimulates cell locomotion. Overexpression of CD44 and RHAMM was detected in supratentorial gliomas as well as diffuse intrinsic pontine glioma (DIPG). HA/CD44 as well as HA/RHAMM cross-talks resulted in glioma cell adhesion, mechanosensing, as well as invasive motility. RHAMM was overexpressed in DIPG and may contribute to the diffuse growth pattern and invasion in DIPG just like other forms of gliomas other than CD44. BEHAB/brevican could be a potential biomarker for glioma detection as well as its progression or malignancy.

INTRODUCTION

Hyaluronan (hyaluronic acid = HA) is ubiquitously present in the extracellular matrix (ECM) of several mammalian tissues[1]. It is a non-sulfated glycosaminoglycan (GAG) of high molecular mass made up of repeated glucuronic acid or N-acetylglucosamine disaccharide components[1-3]. HA is the key constituent of the brain ECM and is particularly enriched in white matter tracts[4,5].

CD44 and RHAMM are both receptors for HA. HA is the mediator of motility as well as mediator of adhesion molecules on the cellular surface[4]. CD44 is a universally expressed cell surface glycoprotein and it is intricate in cell stimulation, cell-cell adhesion as well as cell substrate inter-communication[4,6-9]. It has been postulated that, CD44 has adhesive characteristics instead of the assumed motility functions[10,11].

RHAMM interconnects with HA to stimulate cell locomotion[10,12]. Nevertheless, CD44 interconnecting with HA also triggers cell migration [10,13]. Furthermore, RHAMM appears to be a very crucial controller of cell motility. It has been demonstrated that, in quiescent cells, RHAMM expression is very low. Nevertheless, RHAMM expression becomes obviously upregulated during cell migration or after cytokine stimulation or cell transformation[10]. Kim et al established that, secretion of CD44 was sturdily detected in neurons of normal human brain via *in situ* hybridization while CD44 was extremely secreted by glioma margin as well as the core of gliomas [10].

Although overexpression of CD44 and RHAMM has been detected in supratentorial gliomas[14-16] as well as diffuse intrinsic pontine glioma (DIPG)[17]. Exploiting the role of HA as well as its receptors will add up significantly to the pathological as well as therapy for malignant glioma, because the key course of glioma invasion is through the white matter tracts.

Hyaluronan or Hyaluronic Acid

The high-molecular-weight GAGs, HA, constitutes a substantial percentage of the brain ECM[18-20]. It has a

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huge molecular weight of about 103–104 kDa with about 10,000 or more disaccharide repeating entities[20,21]. HA is synthesized at the innermost surface of the plasma membrane as a free linear polymer without any protein core[21]. Nevertheless, it lacks sulfate groups or uronic acid residues[19]. These features above make HA distinct from other GAGs. HA aids in the migration of several types of tumor cells, and augmented synthesis of HA by tumor cells or tumor-associated fibroblasts was linked to cell migration as well as metastasis[2,22]. HA ability to bind sizeable quantities of H_2O, generates hydrous networks in the extracellular matrix which accelerate cell migration[23].

In the brain interstitium, the high H_2O binding capability of HA enable it regulate the H_2O content of the brain[2]. Studies have shown that, HA partakes in several physiological as well as pathological processes [21,24]. Studies have also proven that, HA is capable of regulating tissue homeostasis, structure integrity as well as tissue cohesion in normal brain ECM[21, 24]. In glioma tissues, HA is capable of simulating intercommunications in the cytoplasm resulting in the triggering of cell proliferation, motility as well as invasion[24,25].

It is established [18] that, HA binds to cell surface receptors like CD44, RHAMM as well as intercellular adhesion molecule-1 (ICAM-1) to stimulate ECM configuration as well as cell matrix intercommunications. It is further proven that; tumors of glial origin have higher quantities of HA which accelerates their invasive capabilities[18,24]. Several studies have also proven that, full-length HA is anti-angiogenic, while oligosaccharides of HA initiate angiogenesis via the triggering of protein kinase C alpha (PKCα) as well as SRC [26-28].

CD44

CD44, a transmembrane glycoprotein (P-glycoprotein 1) is encoded by a single gene on the locus of chromosome 11p13[2,7,29]. CD44 has a molecular weight of 85–200 kDa and it is the most essential HA-receptor ubiquitously secreted by all the body cells[29,30]. CD44 also belongs to the immunoglobulin receptor super family. Perschl et al demonstrated that, several CD44 domains on the cytoplasm can stimulate and enhance HA binding in the cell [31]. Studies have proven that, CD44 intracellular domain compose of a short-tail and long-tail conformation with nuclear localization as well as mediation of transcription [29,32,33].

Functionally, CD44 is divided into four distinctive regions such as, the amino terminal, the stem structure, the transmembrane as well as the cytoplasmic regions[18,34]. The amino terminal region connects to the ECM components via HA as well as other GAGs. The stem structure region often attaches to the amino-terminal as well as the transmembrane regions[35]. The transmembrane region is possibly accountable for the connection between the CD44 and the lipid bundles[36]. The cytoplasmic region of CD44 is linked to the cytoskeleton through ankyrin and other proteins that are essential for cell adhesion as well as motility[37,38].

Apart from the typical form of CD44, numerous splice variations encoded by mutable exons v1–10 (CD44v1–10) has be recognized depending on the cell differentiation as well as activation state[18]. These CD44 variants are capable of binding to numerous growth factors such as vascular endothelial growth factor (VEGF), heparin-binding basic fibroblast growth factor as well as heparin binding epidermal growth factor[29,39]. It is proven that, CD44 is capable of interacting with molecules like collagens, laminins as well as fibronectin *in vitro* [39]. It is further proven that, both the extracellular as well as the intracellular constituents of CD44 stimulate cell migration. CD44 also interacts with numerous regulatory mediators resulting in the triggering of cell signaling pathways[18].

Studies have shown that, CD44 interacts with matrix metallopeptidases (MMP)-mediated matrix degradation resulting in tumor cell growth, migration as well as invasion (Figure 1a) [18,34]. Also, there is evidence suggesting that, the expression of CD44 correlates well with invasion potential of GBM[18,34]. Studies have demonstrated that, MMP-9 and CD44 interaction in mouse and human tumor cells led to the localization of MMP-9 activity on the cell surface[29,40]. Studies have further indicated that, CD44 is secreted by endothelial cells which in turn regulates the formation of new blood vessels [29,40]. In affirmation, inhibition of CD44 led to impaired formation of vessel-like complexes [29,41]. Also, the utilization of CD44 in tumor angiogenesis was increased due to its ability to bind to restrained HA[29,42].

Receptor for Haluronate-mediated Motility (RHAMM)

RHAMM was initially recognized as a portion of a multimeric HA receptor complex (HARC) that intermediated HA-stimulated motility of H-RAS-transformed fibroblast [4]. Nevertheless, Hofmann et al proposed the name RHAMM be changed to intracellular hyaluronic acid binding protein (IHABP) because, they found out that, full-length cDNA for RHAMM encodes a 95 kDa protein which was clearly dissimilar from the protein 'RHAMM'[43]. Studies have demonstrated that, RHAMM is a HA-binding protein that is secreted by cell surface, the cytoplasm, the cytoskeleton as well as the nucleus[1,22].

Studies have shown that, RHAMM is an essential part of HA that facilitate locomotion of tumor cells[13, 44]. Nevertheless, the mechanism involved in this process is very complex and cannot be exclusively elucidated by only HA binding[13]. Moreover, the hypothesis that RHAMM binds to extracellular HA has been questioned based on the findings that, the activities of RHAMM solely transpires intracellularly[13]. A body of evidence indicated that, the communication between HA and RHAMM triggers several cellular signaling pathways involving PKC, focal adhesion kinase (FAK), mitogen-activated protein kinases (MAPKs), nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), RAS, phosphatidylinositol kinase (PI3K), tyrosine kinases (TKs) as well as cytoskeletal factors (Figure 1b)[18,36,45,46].

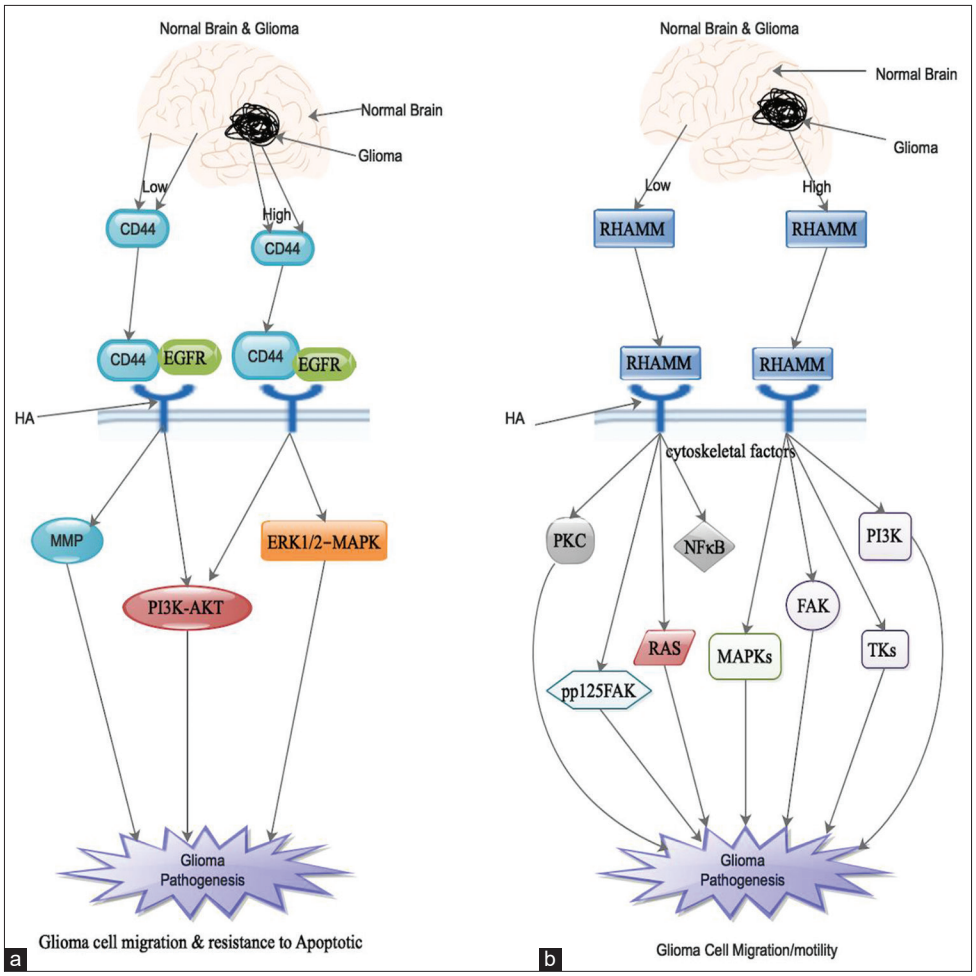

Figure 1. a & b showing the binding of CD44 and RHAMM to HA. The various pathways via which the interactions trigger glioma pathogenesis have illustrated in a & b.

Furthermore, signal transduction cascade via protein tyrosine phosphorylation of several intracellular proteins involving focal adhesion kinase (pp125FAK) has been implicated as the mechanism via which RHAMM mediates cell motility (Figure 1b) [4,16]. Also, RHAMM regulates MAPKs like the extracellular signal-regulated protein kinase (ERK) which is a kinase with dual specificity that controls secretion of proteins pertinent to tumorigenesis, proliferation, as well as motility[4,47].Therefore, CD44 as well as RHAMM perhaps have interrelated roles. Nevertheless, a body of evidence show that, interactions of HA with CD44 as well as RHAMM are obligatory for tumorigenesis as well as tumor progression[18,36,46].

Intercellular Adhesion Molecule-1

Intercellular adhesion molecule-1 (ICAM-l) is another surface protein receptor for HA[48]. Nevertheless, a body of evidence suggested that, ICAM-l binds through hexamethylene receptors and not HA to sepharose matrix [49]. Furthermore, ICAM-l was elutable with HA oligosaccharides, making it seemly look like a receptor for HA[49]. Gingras et al demonstrated that, ICAM-1 was extremely secreted by GBM cells [50]. They suggested that, augmented secretion of ICAM-1 in GBM may bestow a novel potential cell-cell interactions that enriches tumor progression or invasion into surrounding brain[50]. New and modified experiments are still needed to explore more on the receptor capabilities of ICAM-1 on HA.

Hyaluronan and Cell Migration in Glioma

HA is predominantly well secreted by the white matter of the central nervous system (CNS). Also, GFAP positive astrocytes have proven to secret high amounts of HA[4,15]. Another body of evidence showed that, HA upsurges migration of glioma cells through up-modulation of osteopontin[4]. Hayen et al established that GBM cells invasion in porous gels made-up of thick fibrin fibers was faster while invasion in compact gels made-up of thin fibers was slower[2]. They conclude that porosity and not fiber stability was responsible for 3D GBM cell locomotion[2].

Ideally, three distinctive mechanisms probably explanations the stimulation of tumor cell migration in turbid fibrin clots[2]. The augmentation of network stability by thicker fibers which offer stronger tensile forces against moving cells resulting in faster migration[2,51]. Also, augmented permeability or porosity of turbid clots may decrease the requisite for cell-derived proteolytic action. Furthermore, fibrin fibers of different thickness may lead

to distinctive integrin-clustering as well as outside-inside signaling resulting in augmentation of cell migration through the triggering of intracellular signaling cascades[2,52]. A body of evidence indicates that, silencing RHAMM/HA communications results in blockage of cell growth as well as migration[53]. On the other hand, silencing antibodies to CD44 did not change astrocytoma motility[14].

Hyaluronic Acid Receptors and Glioma

HA is much more copious in the ECM of gliomas than in normal brain[19,26]. This means that the overexpression of HA in glioma cell could explain the malignant invasive behavior of glioma cells in brain parenchyma[26]. Kim and Kumar demonstrated that GBM differently secret peculiar HA/CD44-related genes relative to normal brain tissue[26]. They indicated that, HA/CD44 cross-talk resulted in GBM cell adhesion, mechanosensing, as well as invasive motility[26].

A body of evidence suggested that, elevated CD44 levels was associate with mesenchymal subtype of GBM which depicts poor prognosis[4]. Contrarily, Valkonen et al indicated that HA/CD44 did not depict prognosis or any established histopathological parameters in their study[14]. They specified that augmented HA synthases 2 (HAS2) immunostaining concentration was a negative prognostic factor for astrocytomas and correlated appreciably with reduced survival of patients both in univariate as well multivariate analyses[14]. Nevertheless, majority of astrocytomas are histopathologically malignant tumors with very poor prognosis[14].

Several studies have demonstrated that adhesion, spreading, as well as migration of astrocytoma cells are intermediated by cell-cell and cell-matrix adhesion molecules secreted on the astrocytoma cell surface[4, 54-56]. Several studies have found CD44 to be linked with cell-cell adhesion, cell adhesion to ECM, cell migration, as well as cancer development and metastasis[12,57-59]. Nevertheless, excessive quantities of CD44s secretion have been observed in primary human gliomas as well as human glioma cell lines[4, 60-62]. Studies have demonstrated that, CD44 is secreted by astrocytes, mesenchymal cells, epithelial cells, as well as Schwann cells[63-65]. Rutka et al established that, in their study involving invasive astrocytoma cell lines, CD44 was up-regulation[66].

Several studies have exhibited that, CD44 is confined closer to epidermal growth factor receptor (EGFR) and triggers ERK1/2-MAPK as well as PI3K-AKT pathways resulting in GBM cell migration as well as resistance to apoptotic (Figure 1a)[4,67-69]. Xu et al established that, CD44 is elevated in human GBM and that silencing of CD44 decreases GBM growth in vivo via the blockade of glioma cell proliferation as well as stimulating apoptosis[53]. Kim and Kumar reveal that CD44 is capable of facilitating mechanotransductive signaling[26]. They further indicated that, CD44 enhance matrix adhesion as well as 2D motility without generating mature, vinculin-positive focal adhesions. Also, they specified that, glioma cell adhesion, spreading, as well as 2D motility are all sensitive to HA stiffness via CD44. They identified two adhesion systems which support robust 2D motility. CD44-centered adhesions which are associated with extension of small, short-lived activities, and integrin-centered adhesions are linked to broad lamellipodia as well as more purposeful tenacious migration[26].

RHAMM was initially recognized as a fragment of a multimeric HARC [4,11]. Nagy et al demonstrated that, RHAMM was confined to the tips and along the length of neurites[70]. They observed that anti-RHAMM antibodies demonstrated domain-specific blockade as well as triggering of neurite migration in neuronal cultures[70]. They used PC12, NG108-15, and primary neurons to prove that, RHAMM was fundamental for neurite motility as well as migration[70]. Turley et al utilized newborn rat brain cultures as well as two rat immortalized rat brain cell lines to demonstrate that, RHAMM was immunolocalized to the cell surface, punctae, as well as to the plane connecting substrata of astrocytes *in vitro*[71].

The ability of RHAMM to influence astrocyte motility was determine by its localization to cell activities which was proven by the ability of anti-RHAMM antibodies to block astrocyte motility, as well as the mitigation of astrocyte motility via a peptide analogous to the HA-binding domain of RHAMM (Figure 1b) [71]. Specific RHAMM variants were capable of binding fervently to HA following methylpyridinium chloride precipitations and RHAMM soluble peptide blocked glioma cell line proliferation in a dose-dependent fashion[4].

Akiyama et al observed that, RHAMM secretion in malignant gliomas, medulloblastoma cell lines as well as solid tumor specimens secreted different RHAMM variants[4]. Non-neoplastic human brain as well as normal human astrocytes cultures demonstrated their lack of secretion of the RHAMM variants as well as their paucity of secret RHAMM[4]. Studies have shown that, RHAMM intermediates HA-determined migration of neuronal cells as well as numerous varieties of cell in the body[70,72]. Zang et al demonstrated that RHAMM controlled mitogen-activated protein kinases like ERK[47]. ERK is a kinase with duplex specificity that regulates the secretion of proteins significant for tumor development, proliferation, and motility[47].

Hall et al demonstrated that RHAMM/HA cross-talk triggered tyrosine phosphorylation of numerous proteins such as FAK leading to the stimulation of focal adhesion elevation as well as facilitation of cell motility (Figure 1b) [16]. Studies have shown that, ECM-stimulated aggregation of integrins resulted in FAK phosphorylation in human astrocytoma cells[4,56]. It is worth noting that whereas blockade of RHAMM obviously decreases astrocytoma motility, motility was not totally stopped; signifying that, other modules of cell surface receptors like integrins as well as cell adhesion molecule also facilities the motility of astrocytoma cells[4]. Akiyama et al observed an ascending secretion of RHAMM and CD44 amongst gliomas[4]. High-grade gliomas secreted more RHAMM and CD44 than lower grade gliomas or

normal human astrocytes or non-neoplastic specimens of human brain[4].

Akiyama et al demonstrated that high-grade gliomas such as GBM and anaplastic astrocytoma showed robust secretion of RHAMM protein, while low-grade gliomas exhibited poorer secretion of RHAMM[4]. They further established that non-neoplastic surgical specimen rich in astrogliosis exhibited robust RHAMM secretion, while a specimen of non-neoplastic, non-gliotic human brain secreted less amounts of RHAMM[4]. A body of evidence indicates that over-secretion of RHAMM is a potential marker for cancer advancement as well as poor outcomes[73]. Kim et al demonstrated that the secretion of RHAMM was greater in invaded cells than in tumor core[10]. They concluded that, RHAMM significantly partook in glioma invasion than CD44. They further indicated that, both of CD44 and RHAMM were over-secreted at invaded cells surrounding tumors. This means that, CD44 and RHAMM participated in local invasion of glioma[10].

Hyaluronic Acid Receptors and Diffuse Intrinsic Pontine Glioma

DIPG is a fatal brain cancer that arises in the brainstem, with no effective treatment and 100% mortality. It comprises nearly 20% of all brain tumors in children[74] and >90% of children with DIPG died within 1 year of diagnosis [74-76]. They constitute about 75-80% of brainstem tumors in children. There is currently no improvement in prognosis since the first case was detected about five decades ago[74]. Also, the outcome of DIPG is appreciably poorer than other brainstem tumors in pediatric patients[74]. Due to the rarity of this disease, few researchers have directed their attention towards it in the past several decades.

Lan et al demonstrated that, for the first time, RHAMM, one of the HA receptors, was overexpressed in DIPG, which might predict a novel therapeutic target[17]. Their study revealed that, the expression levels of CD44 in DIPG and NDBG were similar. So, CD44 might not be an important factor resulting in the difference in growth pattern and invasion between DIPG and NDBG. Nevertheless, because normal brainstem tissue cannot be obtained for its important functions, they were not able to compare the expression level of CD44 in brainstem glioma with that in normal brainstem tissue[17]. Further studies are necessary to detect whether CD44 is a potential therapeutic target.

In their study, qPCR, western blotting analysis and immunofluorescence staining revealed that, the expressive level of RHAMM was high in DIPG samples as compared to non-diffused brainstem glioma (NDBG), which might predict a novel therapeutic target[17]. Their study showed that the expression levels of CD44 in DIPG and NDBG were similar while the expression levels of RHAMM protein were higher in DIPG as compared to the RHAMM expression levels in NDBG which indicated that diffuse growth pattern and invasion in DIPG might be partly resulted from overexpression of RHAMM[17]. The sample size in this study were very small and the failed to identify of the exact signaling mechanism via which CD44 and RHAMM may exert impact on these glioma types[17]. Further studies are therefore needed in this direction.

Brain Enriched Hyaluronan Binding (BEHAB) as a Biomarker for Gliomas

BEHAB/brevican protein comprises of a HA binding domain in its N terminus as well as an epidermal growth factor-like repeat, a C-type lectin-like domain, as well as a complement regulatory protein-like domain in its C terminus[18,77,78]. Apart from HA binding via the N-terminal domain of BEHAB/brevican, two other binding connections have been identified. BEHAB/brevican binds to the ECM glycoprotein tenascin-R and a subset of membrane sulfated glycolipids via its C-type lectin domain, both via calcium-dependent mechanisms[78-81].

A body of evidence indicates that, BEHAB/brevican mRNA secretion upsurges during the entire rat brain development and plateaued at maturity[79,82]. Studies have shown that, regulation of cell adhesion, neurite outgrowth as well as synaptic plasticity are key functions of BEHAB/brevican during normal brain development[79,83,84]. Nevertheless, in gliomas as well as brain injury, BEHAB/brevican levels were elevated as well as demonstrated to partake in the augmentation of glial cell motility[79,80]. It is affirmed that, gliomas secrets distinctive BEHAB/brevican isoforms and the processing of these explicit isoforms are essential for its pro-invasive functions[85].

A body of evidence suggested that, BEHAB/brevican amasses at the invasive borders and it is linked to high infiltrative profiles in experimentally triggered tumors[86]. Moreover, BEHAB/brevican up-regulation correlates well with short survival periods of patients with high grade gliomas and up-regulation as well as cleavage of BEHAB/brevican can increase the aggressiveness of glial tumors[23, 85]. It is affirmed that, BEHAB/brevican triggers EGFR which in turn triggers the production of cell adhesion molecules and accelerate fibronectin microfibril accumulation in the cell membrane[86].

A study has shown that, the influence of BEHAB/brevican on glioma cells motility was dependent on EGFR signaling as well as fibronectin-dependent adhesion leading to augmented secretion of cell adhesion molecules[23]. Several studies demonstrated elevation of brain-specific isoforms of BEHAB/brevican which also correlated well with peritumoral invasion capabilities of gliomas[23,77,85,86]. Therefore, BEHAB/brevican is a potential biomarker for glioma detection as well as its progression or malignancy.

CONCLUSION

HA is much more abundant in the ECM of gliomas than in normal brain. Also, CD44 and RHMAMM are receptors on the ECM which have proven to partake in facilitating glioma pathogenesis. These receptors are overexpressed in the glioma microenvironment. RHAMM was overexpressed in

DIPG and may contribute to the diffuse growth pattern and invasion in DIPG just like other forms of gliomas. BEHAB/brevican could be a potential biomarker for glioma detection as well as its progression or malignancy.

Abbreviation List

Brain enriched hyaluronan binding = BEHAB, Central nervous system = CNS, Extracellular matrix = ECM, Extracellular signal-regulated protein kinase = ERK, Epidermal growth factor receptor = EGFR, Focal adhesion kinase = FAK, Glioblastoma = GBM, Glycosaminoglycan = GAG, Hyaluronan/hyaluronic acid = HA, HA receptor complex = HARC, HA synthases 2 = HAS2, Diffuse intrinsic pontine glioma = DIPG, Intercellular adhesion molecule-1 = ICAM-1, Intracellular hyaluronic acid binding protein = IHABP, Matrix metallopeptidases = MMP, Mitogen-activated protein kinases = MAPKs, Nuclear factor kappa-light-chain-enhancer of activated B cells = NFκB, Non-diffused brainstem glioma = NDBG, Receptor for hyaluronate-mediated motility = RHAMM, Protein kinase C alpha = PKCα, Phosphatidylinositol kinase = PI3K, Vascular endothelial growth factor = VEGF.

Disclosure

The authors report no conflicts of interest in this work.

Author contributions

All authors contributed toward literature search, drafting and critically revision of the paper and agree to be accountable for all aspects of the work.

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