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Brain sweet brain

Importance of sugars for the cerebral microenvironment and tumor development

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ABSTRACT

The extracellular matrix (ECM) in the brain tissue is a complex network of glycoproteins and proteoglycans that fills the intercellular space serving as scaffolding to provide structural framework for the tissue and regulate the behavior of cells via specific receptors - integrins. There is enormous structural diversity among proteoglycans due to variation in the core protein, the number of glycosaminoglycans chains, the extent and position of sulfation. The lectican family of proteoglycans interacts with growth factors, hyaluronan and tenascin forming a complex structure that regulates neuronal plasticity and ion homeostasis around highly active neurons. In this review, we will discuss the latest insights into the roles of brain glycoproteins as modulators of cell adhesion, migration, neurite outgrowth and glial tumor invasion.

Key words: glycoproteins, extracellular matrix, brain microenvironment, glioma.

Cérebro doce cérebro: importância dos açúcares para o microambiente cerebral e o desenvolvimento tumoral

RESUMO

A matriz extracelular (ECM) no tecido cerebral é formada por uma rede complexa de glicoproteínas e proteoglicanas que preenchem o espaço intercelular participando como estrutura de sustentação do arcabouço tecidual regulando a função celular por interações com receptores específicos - as integrinas. Existe enorme diversidade estrutural entre as proteoglicanas, devido à variação na proteína central (core), à quantidade de cadeias de glicosaminoglicanas, ao grau e posição de grupamentos sulfato na molécula. As proteoglicanas lecticanas interagem com fatores de crescimento, com hialuronana e tenascina formando uma estrutura complexa regulando a homeostase de íons e a plasticidade neuronal. Neste artigo de revisão serão apresentados dados relevantes da literatura sobre o papel das glicoproteínas no microambiente do tecido cerebral, como moduladores da neuritogênese, da adesão, migração celular e invasividade de células tumorais de origem glial.

Palavras-chave: glicoproteínas, matriz extracelular, microambiente cerebral, glioma.

The brain presents restricted well-defined stromal space. A large proportion of the brain volume consists of space between neurons and astrocytic processes filled with extracellular matrix (ECM) components that influence neuronal communication and regulate plastic changes protecting neurons and synapses against damage. The brain ECM is unique in composition and organization as it contains relatively small amounts of fibrous proteins but high amounts of carbohydrates either bound to proteins forming proteoglycans (PG) or unbound in the form of hyaluronan which are abundant in the brain parenchyma. Conversely, the cerebral vascular basement membrane surrounding blood vessels contains type-IV and type-V-collagens, fibronectin, laminin, vitronectin and heparan-sulfate proteoglycans[1].

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Hyaluronan or hyaluronic acid is a multifunctional glycosaminoglycan (GAG) synthesized as a large negatively charged linear polymer by distinct hyaluronan synthases[2]. Hyaluronan organizes the structure of the pericelllular matrix by assuming variety of conformations, from extended chains to condensed rods, helical coils, networks and stacks[3]. The binding to specific cell surface receptors such as CD44 or RHAMM (receptor for hyaluronic acid mediated motility)[4] activates pathways that regulate biological properties of the ECM and tissue function such as cell proliferation, differentiation, adhesion and motility, transport of ions, solutes and nutrients. A synergistic interaction of growth factors (FGF, VEGF, PDGF) with high-affinity receptors stimulates endothelial cell proliferation, vasculogenesis and angiogenesis which is essential for maintenance of stromal integrity during CNS development[5,6]. However, cells that express high affinity receptors but lack surface heparan sulfate proteoglycans do not respond to the stimulus.

PGs are formed by a core protein with one or more covalently attached GAG chain conferring a high negatively charged molecule owing to the presence of acidic sugar residues and/or modification by sulfate groups. The diversity of PG is dependent on differential expression of genes encoding core proteins, alternative splicing and variations in the length and types of GAG side chains. Glypicans and syndecans constitute two families of transmembrane proteoglycans carrying heparan sulfate side chains present in large amounts in the central nervous system. PGs serve as cofactors and regulators of growth factors influencing cell adhesion, neurite outgrowth, ECM assembly and tumor cell invasion[7,8]. Heparan sulfate proteoglycans affect directly the activity and aggregation of AMPA receptors, and also interfere with learning and memory by induction or maintenance of long-term potentiation (LTP). Chondroitin sulfate PG (CSPG) is expressed in regions of the developing brain influencing axonal sprouting and stabilization of synapses, is also abundant postnatally in the cerebellum and hippocampus but decreases markedly after birth[8,9]. In the mature brain, CSPG is present in the cytoplasm of neurons and astrocytes, and in myelinated and non-myelinated axon fibres, but not in oligodendrocytes[1].

Lecticans are a family of chondroitin sulfate proteoglycans that contain lectin and hyaluronic acid-binding domains acting as linkers to ECM components. Molecular cloning identified four lecticans: aggrecan, versican, neurocan and brevican. Glial cells from central and peripheral nervous system, neural stem cells and neural cells derived from embryonic stem cells produce versican[10]. Versican is up-regulated following injury and at least *in vitro* exhibit inhibitory effects upon neurite outgrowth. Neurocan is expressed in boundaries of active axonal growth during development and especially under pathological conditions at the lesion site[11].

Hyaluronan is widely distributed in gray and white matter but aggrecan- and link protein show characteristic regional distribution patterns in perineuronal nets

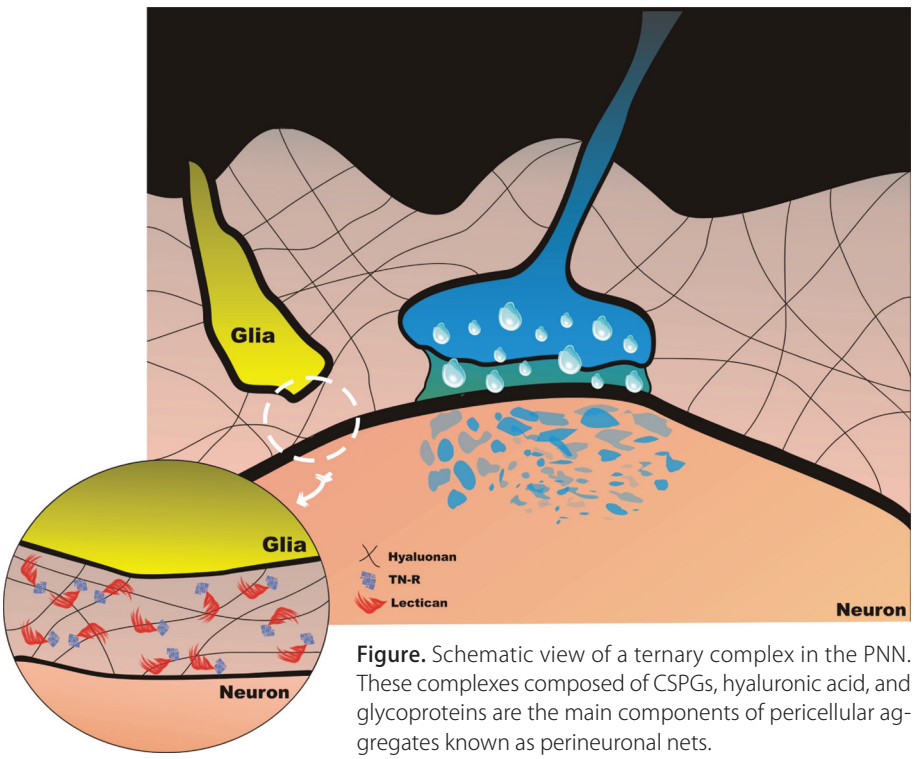

Figure. Schematic view of a ternary complex in the PNN. These complexes composed of CSPGs, hyaluronic acid, and glycoproteins are the main components of pericellular aggregates known as perineuronal nets.

(PNNs) and axonal coats (ACs) encapsulating preterminal fibers and synaptic boutons adapted to the fast processing of sensorimotor activities[6,12]. PNNs are associated with GABAergic projection neurons in the external and internal division of the globus pallidus, the lateral and reticular part of the substantia nigra, and subpopulations of striatal and thalamic inhibitory interneurons expressing parvalbumin, a calcium-binding protein[13]. A dense network of ACs is characteristic of the posterior lateral cell groups (nigrosome 1) of the compact substantia nigra (Figure). PNNs are composed of chondroitin sulfate PGs versican, brevican, neurocan, cat-301 antigen (aggrecan), phosphacan (DSD-1-PG), HA, tenascin-C, tenascin-R, and link proteins forming a structure involved in the regulation of neuronal plasticity and ion homeostasis around highly active neurons[7]. The HA-lecticans-tenascin complex on neuronal surfaces forms a repulsive barrier against axon and dendrites interaction and also development of new synaptic contacts, especially during postnatal life[6]. Neurons ensheathed by PNNs are less frequently affected by lipofuscin accumulation both in normal-aged and Alzheimer's brain tissue[6,14]. Aging and protein aggregation cause deposition of heparan and chondroitin sulfate PGs resulting in loss of protective PNNs and increased susceptibility to cell death. Dying neurons induce inflammation, ECM degradation through proteolytic activity of matrix metalloproteinases and tissue plasminogen activator (tPA), induction of chemotaxis and microglial activation with local cytokine production that amplify neuroinflammation and neuronal death[6,15].

Galectins and collectins (mannose-binding lectins and surfactant proteins) are endogenous glycan-binding proteins present both inside and outside cells, expressing conserved carbohydrate recognition domain (CRD) with a canonical amino acid sequence and affinity for beta galactosides[16,17]. Galectins and its cellular and extracellular ligands mediating cell-to-cell and cell-to-matrix interactions are master regulators of innate and adaptive immune responses under physiological or pathological conditions[16,18]. The extracellular release of galectins is different from classic secretory pathways, as adhesion molecules present high affinity for ECM components and integrins (α1β7/α7β1; CD11b/CD18) important for stabilization of lectin activity, but free Gal molecules are rapidly oxidized and inactivated in the nonreducing extracellular environment[18,19].

Gal-1 is widely distributed in the central and peripheral nervous systems (pia mater, the choroid plexus, the pineal gland, reactive astrocytic and Schwann cells) participating during development in the formation of neural and non-neuronal network. In normal brain tissue, Gal-3 interacts with several neural-tissue-derived glycoproteins (neuronal adhesive glycoprotein L1; N-CAM; myelin-associated glycoprotein), but degree of Gal-3 oligomerization may enhance or inhibit cell adhesion due to steric hindrance. In normal brain tissue Gal3 is highly expressed in normal astrocytes and endothelial cells but completely negative in neurons, oligodendrocytes and microglia[20].

Not so sweet: the role of sugars in tumorigenesis

The invasiveness of glioma cells, a major cause of mortality in malignant brain tumors, is greatly influenced by the cellular microenvironment. Hyaluronan (HA) is present at elevated levels in the matrices of gliomas similarly to the embryonic brain matrix. Aberrant interactions between tumor cells and the ECM allow diffuse infiltration of single tumor cells into the brain parenchyma with cells dividing more rapidly on rigid than on compliant ECMs[21,22].

The progression of the malignancy of a tumor is a multistep process that involves cell-cell and cell-ECM adhesion, invasion, migration, and angiogenesis. Primary GBM, commonly overexpress the epidermal growth factor receptor (EGFR) and its ligand-independent mutant EGFRvIII[23] that activates a number of downstream pathways including PI3K-AKT and RAS-MAPK; induce cell proliferation but inhibits apoptosis. Ras signaling is implicated in gliomagenesis[24] a process partly depend on the dynamic interplay of carboxy-terminal domain of the Ras proteins recognized by Gal-1, Gal-3 and cGMP phosphodiesterase delta[25]. Expression of K-ras, a brain specific isoform of ras and MAPK pathway leads to enhanced transcription of ECM proteins and cytoskeletal rearrangement thus promoting glioma invasiveness[26]. In addition K-Ras-GTP interaction with Gal3 causes a conformational change in the molecule leading to activation of Raf, PI3K pathways but inhibiting ERK signaling and resistance to apoptosis besides modifying biological functions of the tumor suppressor gene *TP53* leading to loss of p53 functionality and chemoresistance[16,27]. Hypoxia confers chemo and radioresistance to tumor cells, modulates the unfolded protein response (UPR) during endoplasmic reticulum (ER) stress, triggers p53 accumulation and activation, and acts as potent inducer of Gal-1 and Gal-3 glycosylation, accelerating malignant progression especially of diffuse astrocytic tumors[2,28].

Gal-1 is involved in several of the steps of malignant progression, mediating tumor cell adhesion to ECM proteins and homotypical cell adhesion and also promoting cell detachment and migration[21]. Gal-1 promotes cell adhesion by increasing crosslinking of glycoproteins (integrins) with carbohydrate moieties of ECM components; increases motility and invasiveness because activates expression of RhoA, protein involved in reorganization of actin cytoskeleton; and the interaction with *Ras* genes which are frequently mutated in human tumors promote malignant transformation[16]. Gal-1 is a major receptor for

the carbohydrate portion of the ganglioside GM1 exposed on the surface of human neuroblastoma cells. The pattern and overexpression of Gal-1 in tumors and surrounding tissue is often considered as a sign of malignant progression but the apparent paradoxical effects on cell growth dependent mainly on the cell type, activation status and characteristics of the molecule (monomeric versus dimeric, or intracellular versus extracellular forms)[20,29]. In addition, Gal-1 is a soluble inhibitory factor also involved in mechanisms of tumor escape by inducing apoptosis of effector T cells, inhibiting the Fas death receptor and perforin / granzymes exocytosis pathways used by cytotoxic T cells to kill tumor cells[18,30]. High serum levels of Gal-1, correlates with aggressiveness of tumors and acquisition of metastatic phenotype and poor prognosis for the patient[29,31]. The level of Gal-3 expression by astrocytes, endothelial cells and macrophages is used to discriminate glioblastoma (GBMs) from anaplastic oligodendroglioma, and pilocytic astrocytoma from diffuse astrocytoma[20,32]. Loss of Gal-3 expression in endothelial cells is associated with glomeruloid endothelial cell proliferation and considered a distinctive feature tumor neovasculature[20]. In contrast, marked expression of Gal-1 occurs under hypoxic conditions associated with necrosis and vascular proliferation, pathological features of the hypercellular zone called pseudopalisades, considered a characteristic formation of malignant infiltrative glioma with poor prognostic[18,33]. The interaction between tumour cells, normal stromal cells and ECM is an essential part of tumor cell invasion and is controlled by different protease systems[1]. The ECM surrounding hypoxic center of pseudopalisades show increased expression of proteolytic enzymes matrix metalloproteinases (MMPs) and the urokinase-dependent plasminogen-activating cascade associated with invasive phenotype, degenerating vessels, and intravascular thrombosis with outward migration of glioma cells[33,34].

Migrating glioma cells express high levels of V0/V1 versican isoforms that interacts via G3 domain with EGFR and beta-1 integrin activating ERK signaling pathways responsible for tumor promoting effects[35,36]. Oligodendroglioma, all grades of astrocytoma, and glioblastoma cells produce high levels of BEHAB/brevican, a secreted chondroitin sulfate proteoglycan with a N-terminal hyaluronan-binding domain, that interacts with fibronectin and further enhance proinvasive capacity of glioma cells[8]. Versican-rich in association with hyaluronan, CD44, tenascin and TGF-β2 in the pericellular matrix of high-grade glioma, exert an anti-adhesive effect facilitating tumor cell migration and invasiveness. Versican splice by tissue proteases produces isoforms that activate ERK cell signaling, promote imbalance between Smad and MAPK pathways responsible for TGF-β tumor promoting effect in high-grade glioma and poor patient survival[36,37]. In addition, abnormal interaction of CD44 hyaluronan receptor and the epidermal growth factor activates plasminogen cascade and promote anchorage-independent growth and invasiveness, hallmarks of the malignant phenotype[9]. Chondroitin sulfate proteoglycans associated with hyaluronic acid, tenascins (TN-X, TN-R, TN-C) and link proteins are also up-regulated especially at the infiltrative tumoral edge of gliomas providing pro-invasive signals to the matrix scaffold by allowing cells to detach rapidly. Increased TN-C expression in gliomas and accumulation around blood vessels correlate with recurrence and tumor progression from grade II to grade III. Moreover, glioma invasion along myelinated fibre tracts of white matter results in spread of tumor cells through the corpus callosum to the contralateral hemisphere[1].

In conclusion, the matrix laid down by cells is a complex molecular scaffolding source of growth factors and proteases that provide survival and danger signals that activate pathways influencing tissue microenvironment and development of neurological diseases. The role of the lecticans as inhibitors of motility in the adult brain tissue contrasts with the proinvasive role in highly aggressive brain tumors and invasion into brain parenchyma.

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