

NG2 Proteoglycan in the Diagnosis, Prognosis and Therapy of Gliomas



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Abstract

The review concerns the state of art of NG2 proteoglycan from its first description to its utilization in glioma therapy. NG2 protein is expressed during development in glia cells committing themselves to a differentiation. It regulates cell proliferation, migration, invasion and neuronal function through a crosstalk with neurons by its extracellular domain. It marks the oligodendrocyte precursor cells, differentiate into mature oligodendrocytes, and also astrocytes. It is expressed also in pericytes and it is involved in their relationship with that endothelial cells.

NG2 distribution in normal central nervous system and in gliomas is discussed, together with its association with Olig2 and PDGFR α .

Recently, it became the focus of attention as a therapeutic target. Various attempts have been made using monoclonal antibodies or RNAi, both in animals and in cell lines. Its ablation resulted in the reduction of glioblastoma cell viability. In children gliomas NG2 distribution is different, but children's brains are very rich in NG2 cells, so that the possibility of a tumor prevention might be hypothesized.

Keywords: NG2; Gliomas; Diagnosis; Therapy

Introduction

Glia cells expressing chondroitin sulfate proteoglycan 4 (CSPG4) are called NG2 (neuron-glia antigen 2) cells and occur in developing as well as in adult brain. They are oligodendrocyte progenitor cells (OPCs) that differentiate into mature oligodendrocytes. Beside supporting neurons and synaptic signaling, they play a role in gliomagenesis and progression and, therefore, they appear as a potential target for diagnosis and therapy of gliomas [1]. In the past, the problem was merely a curiosity, that of glycosaminoglycans in human and in ENU transplacentally induced gliomas [2-4], but today with the description of NG2, Olig2 and platelet-derived growth factor receptor alpha (PDGFR α), it is refined pinning on the origin of gliomas and their therapy.

Discussion

NG2 protein, highly expressed during development and down-regulated during differentiation, beside glia cells, is expressed in pericytes, independently of vasculogenesis and angiogenesis, giving a peculiar significance to their relationship with endothelial cells [5]. NG2 protein is a membrane proteoglycan consisting of a large extracellular, a transmembrane and a short cytoplasmic domain, important for NG2 function [6].

It is activated by ligands through focal adhesion kinase (FAK) and mitogen-activated protein kinase (MAPK) and it regulates cell proliferation, migration, invasion and neuronal function [7] co-acting with PDGFR α . NG2 cleavage by the α -secretase ADAM10 produces in an ectodomain in the extracellular matrix and a C-terminal fragment that is further processed by the γ -secretase to release an intracellular domain. There would be a bi-directional crosstalk between OPCs and the surrounding neuronal network: a novel physiological role for OPCs in regulating information processing at neuronal synapses [8], being the extracellular domain endowed with neuromodulatory properties [9].

NG2 marks OPCs together with PDGFR α ; these cells are distinct from astrocytes and mature oligodendrocytes and for them the term polydendrocytes has been coined [10]. They arise from foci in the wall of the lateral ventricle, including the neurogenic niches of subventricular zone (SVZ) and the hippocampal dentate gyrus [11-13], but it remains to be established whether they give origin to all of oligodendrocyte lineages. They occur, therefore, in the gray and white matter in all brain regions in postnatal development and in adulthood [14]. In the postnatal and adult brain, NG2-glia represents the largest

population of endogenous/resident progenitor cells (4–8% of total cells, depending on the brain region), rapidly reacting, to any type of injury and with a strong potential to repopulate areas of lesion [15-19]. NG2-glia morphology and its functional properties (e.g. membrane channels and receptors) have also been found to be distinct in different brain regions [20-21] as well as the interactions between NG2-glia and other neural cell types [22-25].

Our knowledge on the distribution and functions of NG2 has tremendously increased, but many unresolved questions remain [26]. For example, it is known that, depending on the developmental stage and the brain area, NG2 cells can also produce astrocytes [27-28] or they remain as self-renewing NG2-glia [16]. It has been discussed whether the differentiation of NG2 cells depend on the microenvironment that could condition their ability to form oligodendrocytes or astrocytes [27]. NG2 cells can originate GFAP- and A2B5+ type 2 astrocytes developing later than type 1 astrocytes. Of particular interest is the behavior of NG2 cells as reactive glia after injuries, demyelination, neurodegeneration and as carriers of voltage- and ligand-activated channels [26]. NG2 would not be expressed by multipotent stem cells, but it is up-regulated when stem cells commit themselves to a particular cellular lineage. It is then strongly expressed on partially-committed progenitors that are still proliferative, motile and retain a certain degree of developmental plasticity. Upon terminal differentiation of these progenitors, NG2 expression is down-regulated [6].

Gliomas. NG2+ OPCs have been considered as precursors in glioma development in the adult [29-31]. Only one daughter cell inherits NG2 [30], that is co-expressed with PDGF α for tumor transformation. It is not a marker for a specific cell type, but for an “activated” (as opposed to quiescent) status of cells [6]. Diffuse gliomas express markers characteristic of OPCs, such as NG2, PDGF α and Olig2 [32-34]. OPCs remain widespread in the adult brain [35,36] and they represent the most abundant population of cycling cells in the adult central nervous system (CNS) [35,37] as a pool of cells in which accumulating mutations can lead to gliomagenesis [6,38], as it has been observed in glioma models [39-41]. As a matter of fact, NG2 correlates with the degree of malignancy of gliomas [29,32,42,43].

No pattern of co-expression of NG2, Olig2 and PDGF α has till now been found in glioblastoma (GB), so that it is not known whether the co-expression pattern found in the CNS is recapitulated in GB [44,45]. NG2 expression is widespread in murine and variable in human gliomas [44,45]. Conflicting results were obtained for the distribution of Olig2 [12,46], whereas PDGF α is regularly expressed in II-IV grade gliomas and amplified in GB [47]. Attempts to obtain diagnostic tools [29,48] or to conclude on the GB histogenesis [48] have been made. All the observations are in line with the origin of most gliomas from the subcortical white matter containing

oligodendrocyte precursors expressing NG2, PDGF α and Olig2, recruited experimentally in animals growth factors and in human gliomas [41].

As in normal tissue, NG2 is strongly expressed in pericytes of tumor vasculature with a disruption of the relationship with endothelial cells [32,42,49], even to form tubes without endothelial cells [50]. It has a role in blood vessel development and glioma progression; in its absence the proliferation of pericytes is reduced [51] and the recruitment of endothelial cells is affected [52]. However, in other's experience it has been observed that all PDGF β + pericytes are NG2+, but a small number of NG2+ cells express PDGF β [53].

NG2 cells are found in gliomas conditioning a poor survival. In GB, NG2 would favor cell migration by binding to vascular collagen VI [54] and in pericytes it would favor angiogenesis by sequestering angiostatin [32]. In pediatric gliomas, however, the role of NG2 is not sufficiently known [1].

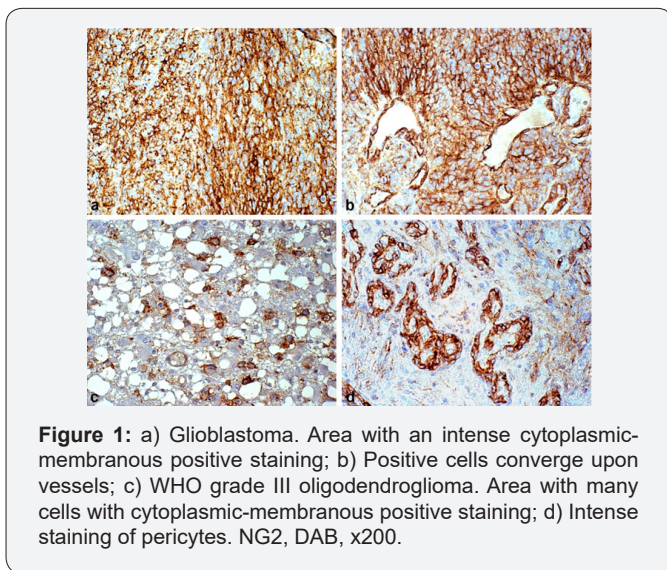
The main function of NG2 is undoubtedly the promotion of cell proliferation and motility, via integrins and growth factors [6]. As for cell motility, beside collagen VI and laminin 2, the contact itself with vessels and neuronal processes seems to be important [55]. In cell proliferation, NG2 intervenes binding fibroblast growth factor 2 (FGF2) and PDGF-AA [56]; as a matter of fact, using anti-NG2 antibody, the proliferation of OPCs is inhibited [6]. The effect on survival would also be supported by $\alpha\beta$ 1 integrin and by the phosphoinositide-3-kinase-protein kinase (PI3K)/Akt pathway [6,43]. NG2-transfected U251 glioma cells are resistant to treatment with tumor necrosis factor alpha (TNF α) and doxorubicin, etoposide and vincristine [43]. There is a relationship between NG2 expression and apoptosis resistance.

By NG2 as biomarker, a NG2+ cell population has been identified with strong proliferative, clonogenic and tumorigenic capacity. It has been shown that 83% of proliferating cells are NG2+ and 50% of NG2+ cells proliferate. NG2+ GB over express genes of mitosis and cell cycling [53].

Recently, therapies based on monoclonal antibodies (mAbs) have gained in popularity given their clinical and commercial success for a variety of malignant diseases [57]. This approach has been applied especially in breast cancer and metastatic melanoma therapy. In gliomas, by targeting NG2 cells by antibodies or RNAi, apoptosis, reduced angiogenesis and invasion have been obtained [58-60]. In experiments on GB animal models, targeting NG2 with the monoclonal antibody mAb9.2.27 and activated natural killer (NK) cells abrogated the tumor growth and prolonged the survival of animals by favoring the establishment of a pro-inflammatory microenvironment [59,60]. Ablating NG2 and GD3A, a ganglioside expressed on developing migratory glia, using a Mab-Zap saporin immunotoxin system, resulted in significant reduction in GB cell viability compared to single epitope targeting and controls [61] In Ethylnitrosourea

(ENU) transplacentally induced gliomas of the rat, NG2 was found to be diffusely distributed in oligodendroglomas and not in cells differentiating into mature oligodendrocytes [62].

All these procedures would be of particular interest in pediatric gliomas, because the child's brain contains a large number of OPCs expressing NG2 [63]. An interesting study demonstrated that 14 mAbs, directed against the ectodomain of human NG2, specifically identify NG2 isoforms in foetal brain and GB tissue, while they are absent in the adult brain [64]. Interestingly, one isoform seems to be exclusive of tumor cells. These results allow to discriminate between phenotype-specific and neoplastic versus non-neoplastic variants of proteoglycans, offering the possibility for more selective immunotherapeutic targeting of brain tumors (Figure 1).



Conclusion

Till now experiments in animals demonstrated the efficacy of NG2 blocking in reducing tumor cell viability and animal survival. The experimentation did not yet transfer to the clinical level, but, since the premises, the possibility that this will happen soon is high.

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