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The regulatory mechanisms of NG2/CSPG4 expression

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Abstract

Neuron-glial antigen 2 (NG2), also known as chondroitin sulphate proteoglycan 4 (CSPG4), is a surface type I transmembrane core proteoglycan that is crucially involved in cell survival, migration and angiogenesis. NG2 is frequently used as a marker for the identification and characterization of certain cell types, but little is known about the mechanisms regulating its expression. In this review, we provide evidence that the regulation of NG2 expression underlies inflammation and hypoxia and is mediated by methyltransferases, transcription factors, including Sp1, paired box (Pax) 3 and Egr-1, and the microRNA miR129-2. These regulatory factors crucially determine NG2-mediated cellular processes such as glial scar formation in the central nervous system (CNS) or tumor growth and metastasis. Therefore, they are potential targets for the establishment of novel NG2-based therapeutic strategies in the treatment of CNS injuries, cancer and other conditions of these types.

Keywords: NG2, CSPG4, Inflammation, Hypoxia, Methylation, Transcription, MiRNA

Introduction

In the last 40 years, many studies have analyzed the structure and functions of neuron-glial antigen 2 (NG2), which is also known as chondroitin sulphate proteoglycan 4 (CSPG4), high molecular weight melanoma-associated antigen (HMW-MAA) or melanoma chondroitin sulfate proteoglycan (MCSP) [1–4]. The NG2 or CSPG4 gene encodes a surface type I transmembrane core protein of ~300 kDa [5]. The extracellular N-terminal domain of this protein is post-translationally modified by chondroitin sulfate glycosaminoglycan chains and disulfide bonds. It also contains putative proteolytic cleavage sites [6]. The function of the extracellular domain fragments is still widely unknown. However, a growing body of evidence suggests that they are involved in the regulation of neuronal networks [7] or endothelial and pericyte functions [8]. The intracellular C-terminal domain of NG2 acts as an acceptor site for the extracellular signal-regulated kinases (ERK) 1/2 and protein kinase C-alpha (PKC-α) as well as a binding site for multi-PZD domain protein 1 (MUPP-1). These interactions activate key signaling pathways involved in cell migration, cell survival and angiogenesis [9, 10].

NG2-mediated signaling has been shown to play an important role in the progression of several tumor types. For instance, elevated NG2 expression is predominantly found in glioblastoma and this correlates with a poor prognosis due to increased NG2-mediated chemo- and radioresistance of the tumor cells [11, 12]. In addition, NG2 serves as a key intermediate of tumor cells with extracellular matrix molecules and

thus crucially determines metastatic formation in soft-tissue sarcoma and melanoma patients [13, 14]. Accordingly, NG2 is a promising target for the development of novel tumor therapies [15–17].

NG2 is also expressed in certain benign cell types. In particular, high levels are detected in NG2-glia of the central nervous system (CNS) [18, 19]. NG2-glia are non-neuronal, non-vascular cells that underlie a complex interplay of epigenetic mechanisms and transcription factors in distinct developmental stages [20, 21]. They are sometimes called polydendrocytes because of their branched morphology or oligodendrocyte precursor cells (OPCs) due to their contribution to the renewal and maintenance of the oligodendrocyte population [22, 23]. Mesenchymal stem cells, osteoblasts, melanocytes, smooth muscle cells and macrophages have also been shown to express NG2 [3, 24, 25]. Finally, the proteoglycan is a typical marker for vessel-surrounding pericytes, which contribute to the stabilization of microvessels, the regulation of capillary blood flow and angiogenesis [26]. Interestingly, the expression pattern of NG2 markedly differs between distinct pericytes depending on the type of analyzed tissue. For instance, only arteriolar but not venular pericytes are positive for NG2 in the mesenteric microvascular network [27, 28]. By contrast, the proteoglycan is expressed in pericytes of all of the microvascular segments in the retina: arterioles, capillaries and venules [29].

These findings indicate that the expression of NG2 underlies a finely balanced regulation dependent on specific cell functions in different tissues. However, which factors are involved in this regulation and how they interact with each other remains elusive. As outlined in the following review, NG2 expression is influenced by inflammation and hypoxia and is intracellularly regulated by methyltransferases, transcription factors and miRNAs (Table 1).

Inflammation

Different NG2-positive cell types in the CNS express receptors for inflammatory cytokines [30–32]. Studies indicate that these cytokines are directly involved in the regulation of NG2 expression and function in response to CNS injuries. For instance, disruption of the blood–brain barrier has been shown to stimulate the release of tumor necrosis factor-alpha (TNF-α), interleukin 1-alpha (IL-1α) and interferon-gamma (IFN-γ) from platelets and other blood components, resulting in increased NG2 levels in OPCs [33]. Gao et al. [34] further detected a higher mRNA and protein level of NG2 in microglial cells following stimulation with lipopolysaccharide (LPS). This was associated with a higher expression of inducible nitric oxide synthase (iNOS), IL-1β and TNF-α, which could be reversed by treatment with NG2 siRNA. In addition, neuroinflammatory disorders, such as autoimmune encephalomyelitis, elevate the expression of NG2 in OPCs, macrophages and CNS-resident microglia, which is mediated by transforming growth factor-beta (TGF-β) [35, 36]. Inhibition of TFG-β activity by decorin [37, 38] or TGF-β1 receptor signaling by SB525334 attenuates this TGF-β-induced NG2 expression [39].

Taken together, these studies indicate that cytokine-mediated NG2 expression is a major response mechanism to various destructive processes in the CNS. In this context, it should be considered that NG2 is an important contributor to glial scar

Table 1 Studies focusing on factors which regulate the expression of NG2/CSPG4

Regulatory factors / treatment		Species	Type of cells or tissue	Analysis	Expression	Reference
Inflammation	TNF-α, TGF-β, IL-1α or IFNγ	Rat	OPC	Protein	↑	[22]
	TGF-β	Mouse	Macrophages, OPC	mRNA, Protein	↑	[24]
	TGF-β	Rat	Cerebral cortex	Protein	↑	[25]
	Decorin	Rat	Spinal cord	Protein	↓	[26]
	TGF-β receptor inhibitor	Rat	Microglia cells	mRNA, Protein	↓	[28]
	LPS	Rat	Microglia cells	mRNA, Protein	↑	[23]
	IL11, LIF	Human	Placental villous tissue	mRNA, Protein	↑	[32]
	Chronic hypoxia (5 d)	Rat	Mesentery	Protein	↑	[35]
Hypoxia	Chronic hypoxia (48 h)	Human	Panc1, HS766T	mRNA, Protein	↑	[39]
DNA Methylation	Methyltransferase inhibitor (5-aza-2′-deoxycytidine)	Human	Melanoma cells	mRNA, Protein	↑	[45]
	Methyltransferase inhibitor (5-aza-2′-deoxycytidine)	Human	Head and neck squamous cell carcinoma	mRNA, Protein	↑	[46]
Transcription factors	Truncated promoter constructs	Monkey	COS cells	Luciferase activity	↑	[49]
	Sp1 siRNA	Human	Keratinocytes	mRNA	↓	[50]
	Pax3	Human	Melanocytes, melanoma cells	mRNA	↑	[54]
	Pax3 siRNA	Human	Melanocytes, melanoma cells	mRNA	↓	[55]
	Egr1 siRNA	Mouse/Human	Astrocytes	mRNA, Protein	↓	[31]
miRNA	miR129-2	Mouse	Neurospheres	mRNA, Protein	↓	[62]

The table lists the species and type of cells or tissue in which the analyses have been performed as well as the level of detection and the observed up- (↑) or downregulation (↓) of NG2/CSPG4 expression

formation [40], during which increased expression levels of extracellular matrix components and chondroitin sulfate proteoglycans, including NG2, form an inhibitory barrier to regenerating axons, blocking their outgrowth in the surrounding tissue [41, 42]. Accordingly, the modulation of this process may be a promising approach to promote neuronal repair after traumatic or inflammation-induced CNS injuries.

In addition, van Sinderen et al. [43] recently analyzed the role of NG2 in the placenta and extravillous trophoblasts. They found that IL-11 and leukemia inhibitory factor (LIF), known to be produced by the placenta in the first trimester [44, 45], stimulate NG2 expression specifically in the placental villi and deciduas. They speculated that these two cytokines stimulate the early differentiation of the cytotrophoblast cells towards the migratory extravillous trophoblast phenotype via the upregulation of NG2 levels.

Hypoxia

Several studies show that NG2 expression may be regulated by hypoxia-induced signal transduction. Under normoxic conditions, NG2 expression is only found in pericytes located along the arterioles and capillaries but not along the venules of adult rat mesenteric microvascular networks [28]. Exposure of these networks to chronic hypoxia is associated with additional expression of the proteoglycan in venular pericytes [46]. This indicates an important function of NG2 in these activated cells during hypoxia-induced angiogenesis and vascular remodeling [46]. Concurrent with this view, Ozerdem et al. [47] found a substantially reduced neovascularization in the ischemic retinas of NG2 knockout mice when compared to wild-type controls.

Hypoxia-inducible factors (HIFs) are the most important transcription factors mediating hypoxic expression of target genes [48]. Accordingly, they may also be involved in the regulation of NG2. Under normoxia, HIFs are constitutively expressed in the cytoplasm with a very short half-life, because they are hydroxylated. This promotes their binding to von Hippel-Lindau protein (VHL), which targets HIFs for rapid proteasomal degradation. Under hypoxia, non-hydroxylated HIFs translocate to the nucleus, resulting in increased target gene expression [49]. Concurrent with these findings, Keleg et al. [50] could demonstrate that NG2 mRNA and protein levels are already overexpressed in the normoxic pancreatic cancer cell lines Panc1 and HS766T, which exhibit mutations of VHL. Exposure of these cells to chronic hypoxia further enhances these high NG2 mRNA and protein expression levels.

DNA methylation

DNA methylation has recently been identified as a major epigenetic mechanism for the regulation of gene expression [51]. It is characterized by the transfer of a methyl group to the 5' cytosine of a CpG dinucleotide by DNA methyltransferases, resulting in the suppression of gene transcription [52]. Promoter methylation of tumor suppressor genes is particularly found during carcinogenesis, indicating that this process induces the development of many types of tumor [53–55]. Interestingly, Luo et al. [56] found that the promoter of the NG2 gene also contains many 5'CpG methylation sites. It has further been demonstrated that reduced methylation of this promoter increases the expression of NG2 in melanoma cell lines, primary melanoma lesions, and head and neck

squamous cell carcinoma [56, 57]. Since high NG2 expression is often associated with elevated multi-drug resistance [11, 12, 58], methylation of the NG2 promoter may thus determine the therapy response and prognosis of many cancer types.

Transcription factors

In addition to DNA methylation, NG2 expression is also regulated by several transcription factors. In 2009, Sellers et al. [59] described the regulatory region (1585 bp) upstream of the mouse NG2 coding sequence in detail. The TATA-box of the NG2 promoter is 1299 bp upstream of the transcriptional start. Putative binding sites for transcription factors are located within this region. As identified by Transcriptional Element System Search (TESS), these transcription factors may include C/EBP, p300, CBP and Sp1. The latter seems to have a particularly crucial role in the regulation of NG2 gene expression. Sellers et al. [59] showed that transfection of COS cells with luciferase reporter gene constructs that contain the NG2 promoter without a TATA-box and the upstream located Sp1 binding sites, results in increased cellular luciferase activity. On the other hand, Leung et al. [60] found that silencing Sp1 downregulates the transcription of the proteoglycan in keratinocytes. These contradictory findings suggest that additional post-transcriptional modifications of the constitutively expressed transcription factor Sp1 determine its function as a transcriptional activator or repressor of NG2 [61].

Paired box 3 (Pax 3) is another transcription factor capable of influencing target gene transcription in a positive [62] or negative [63] manner. However, Pax3 has only been shown to increase the expression of NG2 in melanocytes [64]. Pax3 silencing results in a diminished expression of the proteoglycan in these cells [65]. It has further been reported that TGF-β suppresses the expression of Pax3 in melanocytes via a smad-dependent pathway [66]. Since this growth factor upregulates NG2 levels in CNS-resident microglia [35, 36, 39], this finding indicates that other transcription factors must be involved in TGF-β-induced NG2 expression or that its regulation markedly differs between individual cell types.

The transcription factor Egr-1 is a crucial mediator in ERK-dependent signaling during cell survival, apoptosis and differentiation [67]. ERK phosphorylates and activates the transcription factor Elk-1 [68, 69], which increases the expression of Egr-1 [70]. Egr-1 regulates the expression of different genes encoding for adhesion proteins and cytokines [71]. Beck et al. [42] demonstrated that after cerebral ischemia, reactive astrocytes exhibit high levels of Egr-1. Silencing Egr-1 in these cells diminished the expression of genes that are important for glial scar formation, including NG2. They concluded that this transcription factor may represent a potential target for the modulation of neuronal tissue repair and regeneration.

miRNAs

In the last decade, microRNAs (miRNAs) have been identified as novel, powerful regulators of protein expression. They are endogenously expressed small non-coding RNA molecules that suppress protein expression by interacting with the target messenger RNA (mRNA) [52]. miR129-2 belongs to the group of tumor suppressor miRNAs, because its transcription is downregulated in some types of cancer due to increased

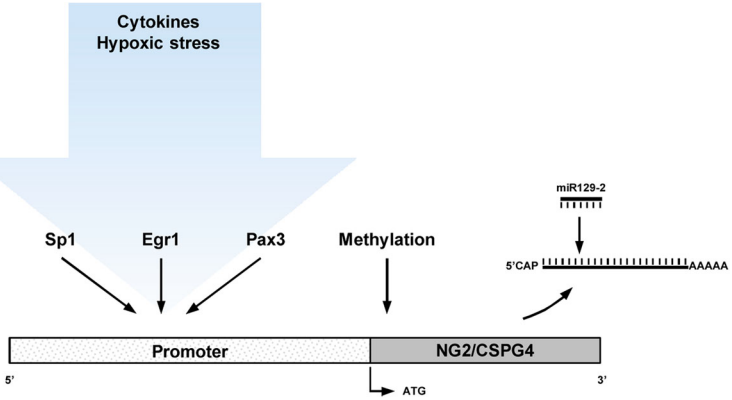

Fig. 1 Known regulatory factors of NG2 (CSPG4) expression. NG2 expression underlies inflammatory cytokines and hypoxic stress and is mediated by methyltransferases, transcription factors such as Sp1, Egr1 and Pax3, and miR129-2

methylation [72]. It was recently reported that overexpression of miR129-2 decreases NG2 levels in diffuse intrinsic pontine gliomas [73]. miR129-2 also suppresses the expression of platelet-derived growth factor receptor-alpha (PDGFR-α) in glioma cells [74]. This receptor stimulates the proliferation of various mesenchymal and glial cells and is one of the most amplified genes in glioblastoma [75]. In addition, interaction of NG2 with PDGFR-α has been shown to promote cell proliferation in response to PDGF [76, 77]. These findings suggest that miR129-2 may represent a promising candidate for NG2-targeting tumor therapy.

Conclusion

Although NG2 is important for cell function and frequently used as a marker for the characterization and identification of certain cell types, there is little knowledge about the mechanisms that regulate the expression of this proteoglycan. The reports discussed here provide the first evidence that this regulation underlies inflammation and hypoxia and is mediated by methyltransferases, transcription factors and miRNAs (Fig. 1). In the future, the identification of additional regulatory factors may further improve our understanding of NG2-mediated cellular functions, such as cell survival, migration and angiogenesis. In addition, it may also contribute to the establishment of NG2 as a novel therapeutic target in the treatment of CNS injuries, cancer and other conditions.

Abbreviations

CNS: Central nervous system; CSPG4: Chondroitin sulphate proteoglycan 4; ERK: Extracellular-signal-regulated kinase; HIF: Hypoxia-inducible factor; HMW-MAA: High molecular weight melanoma-associated antigen; IFN: Interferon; IL: Interleukin; iNOS: Inducible nitric oxide synthase; LIF: Leukemia inhibitory factor; LPS: Lipopolysaccharide; MCSP: Melanoma chondroitin sulfate proteoglycan; miRNA: MicroRNA; MUPP: Multi-PZD domain protein; NG2/CSPG4: Neuron-glial antigen 2; OPC: Oligodendrocyte precursor cell; Pax: Paired box; PDGFR: Platelet-derived growth factor receptor; PKC: Protein kinase C; TESS: Transcriptional element system search; TGF: Transforming growth factor; TNF: Tumor necrosis factor; VHL: Von Hippel-Lindau.

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EA reviewed the literature and wrote the manuscript. BMS was a contributor in reviewing the literature. MDM and MWL revised the manuscript. All authors read and approved the final manuscript.

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