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Nucleolin is a nuclear target of heparan sulfate derived from glypican-1

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ABSTRACT

The recycling, S-nitrosylated heparan sulfate (HS) proteoglycan glypican-1 releases anhydromannose (anMan)-containing HS chains by a nitrosothiol-catalyzed cleavage in endosomes that can be constitutive or induced by ascorbate. The HS-anMan chains are then transported to the nucleus. A specific nuclear target for HS-anMan has not been identified. We have monitored endosome-to-nucleus trafficking of HS-anMan by deconvolution and confocal immunofluorescence microscopy using an anMan-specific monoclonal antibody in non-growing, ascorbate-treated, and growing, untreated, wild-type mouse embryonic fibroblasts and hypoxia-exposed Alzheimer mouse Tg2576 fibroblasts and human U87 glioblastoma cells. In all cells, nuclear HS-anMan targeted a limited number of sites of variable size where it colocalized with DNA and nucleolin, an established marker for nucleoli. HS-anMan also colocalized with ethynyl uridine-tagged nascent RNA and two acetylated forms of histone H3. Acute hypoxia increased the formation of HS-anMan in both Tg2576 and U87 cells. A portion of HS-anMan colocalized with nucleolin at small discrete sites, while most of the nucleolin and nascent RNA was dispersed. In U87 cells, HS-anMan, nucleolin and nascent RNA reassembled after prolonged hypoxia. Nucleolar HS may modulate synthesis and/or release of rRNA.

1. Introduction

Glypicans (Gpc) are a family of cell-surface heparan sulfate (HS) proteoglycans that regulate growth-factor and morphogen signaling [1,2]. The Gpc-1 protein, which is ubiquitously expressed in vertebrate tissues, consists of a large N-terminal α-helical domain containing 14 conserved, disulfide-bonded Cys residues followed by a flexible HS-attachment region and the C-terminal glycosylphosphatidylinositol membrane anchor [3] as shown in Fig. 1A. The flexible region also contains two non-conserved Cys residues that are not disulfide bonded (Cys-SH) and can be S-nitrosylated (Cys-SNO) in a copper-dependent reaction [4,5]. *In vitro*, the SNO groups in recombinant Gpc-1 can catalyze deaminative cleavage of the HS chains at N-unsubstituted glucosamines in a reaction that is induced by ascorbate. This results in the release of HS chains and oligosaccharides containing reducing terminal anhydromannose (HS-anMan) and a Gpc-1 core protein with short HS stubs as shown in Fig. 1B.

In cell cultures, cell surface Gpc-1 is internalized via a caveolin-1-associated pathway, S-nitrosylated and transported to endosomes where the SNO/nitric oxide (NO)-catalyzed autodegradation takes place. Formation of HS-anMan is constitutive in dividing fibroblasts and suppressed in non-dividing ones but can be restored by exogenously supplied ascorbate [6,7].

HS as well as other glycosaminoglycans have been found in various cell nuclei [8–11]. It is not known how HS is transported to the nucleus, nor if it is associated with specific nuclear structures. We have recently shown, by immunofluorescence microscopy using an anMan-specific monoclonal antibody and by [^{35}S]sulfate-labeling and isolation of nuclear HS, that HS-anMan generated by ascorbate treatment of wild-type mouse embryonic fibroblasts (WT MEF) and N2a neuroblastoma cells penetrates the endosomal membrane and translocates to the nucleus. Penetration and transfer was dependent on expression and processing of the amyloid precursor protein. HS-anMan eventually disappeared from the nucleus and was captured in autophagosomes/lysosomes for final destruction [12]. Here, we show that nucleolin is involved in nuclear targeting of HS-anMan suggesting that HS-anMan interacts with nucleoli.

2. Materials and methods

2.1. Cells and reagents

Mouse embryonic fibroblasts (MEF) from wild-type (WT) mice and

Abbreviations: anMan/AM, anhydromannose; DAPI, 4,6-diamidino-2-phenylindole; Gpc, glypican; HS, heparan sulfate; mAb, monoclonal antibody; MEF, mouse embryonic fibroblast; NO, nitric oxide; SH, thiol; SNO, S-nitrosothiol; Tg2576, transgenic Alzheimer mouse; WT, wild-type

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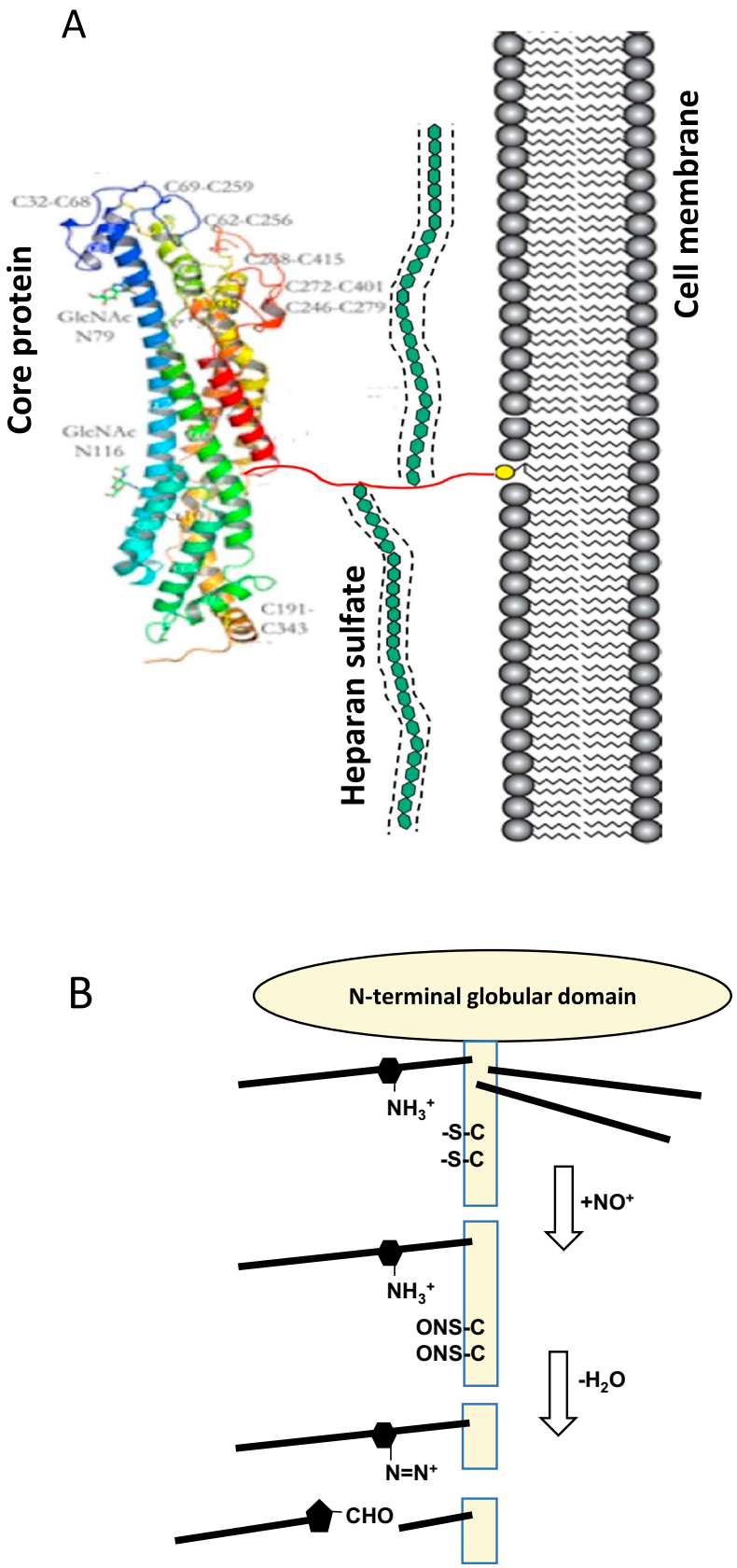

Fig. 1. Structure of the Gpc-1 proteoglycan and the mechanism for SNO/NO-dependent deaminative cleavage of HS. (A) The Gpc-1 core protein consists of a large N-terminal, α-helical, globular domain that contains 7 disulfide bonds (C-C) and two N-linked glycans (GlcNAc), an unstructured stem region to which the polyanionic (——) HS chains (string of green hexagons) are attached and a C-terminus that is anchored to the membrane via a glycolipid (yellow dot). The stem region and the HS chains are not drawn to scale, the latter should extend far beyond the globular N-terminal domain. (B) In addition to the 3 HS chains (———), the C-terminal stem region also contains 2 Cys with free thiols/thiolates ($^{-}$S-C). These can be S-nitrosylated by nitrosonium ions (NO^{+}) in a copper-dependent reaction. The HS chains contain clusters of N-unsubstituted glucosamines ($GlcNH_2/GlcNH_3^{+}$, only one of which is depicted as a hexagon), preferentially located near the linkage region to the protein core. Attraction between $GlcNH_3^{+}$ and the nitrosothiol dipol ($^{\delta-}ONS^{\delta+}$-C) favors an NO-catalyzed reaction that converts the amino group to a diazonium ion (–N=N^{+}). The diazotized glucosamine undergoes a spontaneous ring contraction accompanied by cleavage of the glycosidic bond releasing HS with a reducing terminal anMan residue (pentagon), exposing C-1 as a free aldehyde (-CHO) and leaving behind a Gpc-1 with truncated HS stubs.