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Title: Novel Cleavage-Dependent Interaction of Proteoglycan Receptors with Neuropilin-1

Abstract

Receptor tyrosine phosphatases (RPTPs) play a critical role in regulating signaling events in coordination with tyrosine kinases. RPTPs not only regulate tyrosine phosphorylation of intracellular proteins at the intersection of many signaling pathways but also play key roles in transmitting extracellular signals into the cytosol. The type IIa subfamily of RPTPs (RPTPσ, RPTPδ, and RPTP-LAR) serves as functional receptors for proteoglycan glycosaminoglycans (GAGs).

In this study, we compared the characteristics of RPTPσ and RPTPδ. We found that the extracellular domains (ECDs) of both RPTPσ and RPTPδ distinguished the extent of sulfation on GAG chains based on solid-phase binding assays and Glycan Microarray. Notably, we observed striking differences: (1) RPTPδ possessed a single GAG binding site, whereas RPTPσ exhibited an additional heparin binding site in the juxtamembrane domain; (2) complete cleavage was observed within the ECD of RPTPδ, whereas only partial cleavage occurred in the RPTPσ ECD; and (3) the novel heparin binding site and the potential cleavage site on RPTPσ overlapped.

Identification of the exact cleavage sites within the ECD of RPTPσ and RPTPδ in this study led us to conclude that furin was the responsible enzyme. This conclusion is based on the in vitro cleavage of recombinant RPTPσ and RPTPδ by the recombinant furin (rFurin), and the inhibition of the cleavage by the furin inhibitor in HEK293 cells and no cleavage in the furin-deficient cells. Furthermore, exogenous heparin partially blocked the cleavage of RPTPσ by rFurin, suggesting that heparan sulfate acts as a potential regulator of RPTPσ ECD cleavage.

We discovered that the cleavage products of RPTPσ and RPTPδ bound to neuropilin-1 (NRP1) through their C-terminal end, known as the "C-end Rule [CendR] motif". Solid-phase binding assays revealed that both cleavage products exhibited very high-affinity binding (K_d=~10^{-9}M) to NRP1, with 2 binding sites on RPTPδ and a single binding site on RPTPσ.

Since NRP1 plays a role as a co-receptor in multiple signaling pathways, including VEGF-A and TGFß, we sought to identify the pathways that are potentially affected by the cleavage products of RPTPσ and RPTPδ. Recombinant ECDs of RPTPσ and RPTPδ reduced the proliferation of human umbilical vein endothelial cells (HUVEC) induced by VEGF-A in a CendR motif-dependent manner, showing that the recombinant fragments were biologically active. Further, the exogenous ECD of RPTPδ reduced TGFß-induced transcription in a CendR motif-dependent manner. In contrast, RPTPσ reduced TGFß-induced transcription independently of CendR.

While the ECD cleavage of type IIa RPTP has long been known but often overlooked, our findings shed light on the physiological relevance of this process.