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Research Article

A systematic expression analysis implicates Plexin-B2 and its ligand Sema4C in the regulation of the vascular and endocrine system

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ABSTRACT

Plexins serve as receptors for semaphorins and play important roles in the developing nervous system. Plexin-B2 controls decisive developmental programs in the neural tube and cerebellum. However, whether Plexin-B2 also regulates biological functions in adult nonneuronal tissues is unknown. Here we show by two methodologically independent approaches that Plexin-B2 is expressed in discrete cell types of several nonneuronal tissues in the adult mouse. In the vasculature, Plexin-B2 is selectively expressed in functionally specialized endothelial cells. In endocrine organs, Plexin-B2 localizes to the pancreatic islets of Langerhans and to both cortex and medulla of the adrenal gland. Plexin-B2 expression is also detected in certain types of immune and epithelial cells. In addition, we report on a systematic comparison of the expression patterns of Plexin-B2 and its ligand Sema4C, which show complementarity or overlap in some but not all tissues. Furthermore, we demonstrate that Plexin-B2 and its family member Plexin-B1 display largely nonredundant expression patterns. This work establishes Plexin-B2 and Sema4C as potential regulators of the vascular and endocrine system and provides an anatomical basis to understand the biological functions of this ligand–receptor pair.

Introduction

Semaphorins are a large family of secreted and membrane-associated proteins that were first described as axon guidance cues in the developing nervous system [1,2]. More recently, they have also been recognized as key regulators of organogenesis, the immune system, and cancer [3–5]. Most effects of semaphorins are achieved via activation of transmembrane receptors called plexins [6]. Based on sequence similarities, plexins have been divided into four subfamilies (A–D). The activation of plexins by semaphorins triggers several intracellular signalling pathways that involve small GTPases of the Ras and Rho families [7]. Whereas all plexin family members possess a R-Ras GTPase activating protein (GAP) domain, only B-family plexins mediate the activation of RhoA

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through an interaction with Rho guanine nucleotide exchange factor (RhoGEF) proteins [7]. While Plexin-B1 is activated by semaphorin 4D (Sema4D) [6], Plexin-B2 binds Sema4C and Sema4D with high and low affinity, respectively [8,9], and Plexin-B3 responds to Sema5A [10]. Recently, Sema4A has been reported to bind to all three B-type plexins [11,12].

During mouse development, Plexin-B1 and Plexin-B2 are widely expressed both inside and outside the nervous system, whereas Plexin-B3 expression is restricted to postnatal oligodendrocytes [13,14]. Using genetically modified mice, several recent studies have addressed the biological significance of B-family plexins during mouse development. Plexin-B1-deficient mice exhibit abnormalities in epithelial branching morphogenesis during development of the kidney [15] and defects in migration of GnRH-1 neurons during development of the nervous system [16]. Plexin-B3-deficient mice are devoid of obvious histological and behavioural abnormalities, thereby suggesting that Plexin-B3 is not essential for normal development and function of the central nervous system [17].

Unlike Plexin-B1- and Plexin-B3-deficient mice, which show a rather mild or no obvious phenotype, respectively, mice lacking Plexin-B2 display severe defects in several developmental processes, including proliferation, migration, and pattern formation in the mouse forebrain and cerebellum [8,18,19]. Plexin-B2 knockout mice fail to close the cephalic neural tube and are found with an exencephalic phenotype. In the cerebellum and the developing cortex, Plexin-B2 deficiency results in profoundly altered layering and defective migration and differentiation of cerebellar and cortical neurons.

In contrast to the developing nervous system, very little is known about the functional significance of Plexin-B2 in adult nonneuronal tissues. As a prerequisite to understanding the biological role of Plexin-B2 outside the nervous system, we set out to establish a precise map of the expression of Plexin-B2. In addition, we systematically compared the expression pattern of Plexin-B2 to that of its high-affinity ligand Sema4C to address potential functions of this ligand–receptor pair. In light of the promiscuous interactions between semaphorins and semaphorin receptors [20], with e.g., Sema4D binding to both Plexin-B1 and Plexin-B2 with different affinities [6,9], Plexin-B1 and Plexin-B2 might be functionally redundant in those tissues where their expression patterns overlap. To therefore identify possible sites of mutual compensation, we further extended our expression analysis to Plexin-B1.

This study reveals that expression of Plexin-B2 and its ligand Sema4C is not confined to development but also widely found in the adult mouse. We demonstrate that Plexin-B2 and Sema4C are expressed in discrete cell types of several nonneuronal tissues including endothelial and endocrine cells. Moreover, we show that Plexin-B1 and Plexin-B2 display largely nonredundant expression patterns in the adult mouse.

Materials and methods

Genetically modified mice

The generation of heterozygous knock-in mutant mice expressing a cDNA encoding β-galactosidase (LacZ) targeted into either the *plxnb1*, *plxnb2*, or *sema4C* locus [18,21] and the generation of mice with constitutive global deletion of the *plxnb2* gene [8] have been described in details before.

Analysis of β-galactosidase activity

Tissues were fixed overnight in 0.2% paraformaldehyde in PBS at 4 °C, followed by 2 days of incubation in 30% sucrose in PBS at 4 °C. Tissues were then frozen and cryosectioned at 25 µm. Sections were stained in staining solution (0.1 M PBS pH 7.3, 2 mM $MgCl_2$, 5 mM EGTA, 0.01% sodium deoxycholate, 0.02% NP-40, 10 mM potassium ferricyanide, 10 mM potassium ferrocyanide, 0.5 mg/ml X-gal) at 37 °C overnight in the dark. Subsequently, sections were counterstained with eosin, dehydrated, and mounted in Pertex solution (Medite) using standard protocols. Corresponding wild type tissue was routinely included in every experiment and entirely failed to yield signals.

Immunofluorescence and confocal microscopy

Tissues were fixed in 0.2% paraformaldehyde/PBS at 4 °C overnight and incubated in 30% sucrose/PBS at 4 °C for 48 h. Tissues were frozen and cryosectioned at 25 µm. Sections were postfixed in 4% paraformaldehyde/PBS at 4 °C for 10 min, washed twice in 50 mM glycine in PBS, blocked for 30 min in blocking solution (0.2% Triton X-100, 4% normal horse serum, in PBS) and incubated with primary antibodies in 0.2% Triton X-100, 4% normal horse serum/PBS overnight at 4 °C. Sections were washed three times in 1% normal horse serum/PBS, incubated with secondary antibodies in 1.5% normal horse serum/PBS for 2 h at room temperature in the dark, stained in DAPI solution, washed in 10 mM Tris–HCl, and mounted with Aqua-Poly/Mount (Polysciences). Primary antibodies were Armenian hamster monoclonal anti-Plexin-B2 (clone 3E7, 1:500; eBioscience), rat monoclonal anti-Sema4D (clone BMA12, 1:50; eBioscience), goat polyclonal anti-β-gal (1:500; Cappel), rat monoclonal anti-CD31 (1:200; BD Biosciences), guinea pig polyclonal anti-insulin (1:200; Abcam), rabbit polyclonal anti-glucagon (1:1000; Linco), rat monoclonal anti-CD11b (1:200; eBioscience), mouse monoclonal anti-SMA (1:200; Sigma), and rabbit polyclonal anti-Clara cell secretory protein (1:200; Millipore). Secondary antibodies were Cy3-conjugated anti-Armenian hamster (1:200; Jackson Immunoresearch), AlexaFluor 488-conjugated anti-goat (1:200; Invitrogen), Cy3-conjugated anti-goat (1:200; Jackson Immunoresearch), AlexaFluor 488-conjugated anti-rat (1:200; Invitrogen), Cy2-conjugated anti-guinea pig (1:200; Jackson Immunoresearch), AlexaFluor 488-conjugated anti-rabbit (1:200; Invitrogen), and AlexaFluor 488-conjugated anti-mouse (1:200; Invitrogen). All images were captured on a confocal laser scanning microscope (Leica TCS AOBS) and processed using Leica confocal software.

Results

The expression patterns of Plexin-B2, Sema4C, and Plexin-B1 were analyzed using genetically modified mouse lines expressing the reporter gene product β-galactosidase under the control of the Plexin-B2, Sema4C, and Plexin-B1 promoter, respectively (*plxnb2lacZ*, *sema4ClacZ*, and *plxnb1LacZ*). As the reporter gene cassette encoding β-galactosidase is inserted into the genomic locus of the respective genes, the entire endogenous promoters and