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Regulation of Dendritogenesis in Sympathetic Neurons

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Abstract

In postganglionic sympathetic neurons, the size of the dendritic arbor determines pre-synaptic convergence, which correlates with tonic activity, and aberrant dendritic morphology is associated with disease. There is, therefore, great interest in understanding how dendritic morphology is regulated in these neurons. Early studies established a role for target-derived nerve growth factor (NGF) in regulating the size of the dendritic arbor of sympathetic neurons *in vivo*. However, *in vitro* studies revealed that even in the presence of optimal concentrations of NGF, rat sympathetic neurons cultured in the absence of serum or non-neuronal cells survive and elaborate extensive axonal arbors, but fail to form dendrites. Subsequently, it was discovered that bone morphogenetic proteins (BMPs) trigger cultured sympathetic neurons to extend a dendritic arbor comparable to that of their *in vivo* counterparts. The goals of this chapter are to: (i) summarize these early experiments; (ii) discuss evidence substantiating a role for BMPs in glial-induced dendritic growth *in vitro* and regulation of dendritic growth *in vivo*; (iii) review what is known about the molecular mechanisms by which NGF, BMPs and other factors influence dendritic arborization of sympathetic neurons; and (iv) identify key data gaps in understanding of how dendrites are regulated in sympathetic neurons.

Keywords: afferent input, BMPs, dendrites, neuronal polarity, NGF, p75, reactive oxygen species (ROS), Rit, Smad, STAT, sympathetic neurons, target-derived factors

1. Introduction

Differences in dendritic morphology between neurons are a striking feature of the vertebrate nervous system with important functional implications. The shape of dendrites influences the propagation and integration of postsynaptic potentials [1], and determines presynaptic

convergence [2, 3]. These observations coupled with evidence that aberrant dendritic structure is strongly associated with neurologic disease [4, 5] have generated significant interest in understanding how dendrites are regulated.

Postganglionic sympathetic neurons are a well-characterized model for studying dendrite development and plasticity [6]. The dendritic arbor of these neurons is relatively complex with an average of two to six primary dendrites, depending on the animal species, and multiple orders of branching [7]. In postganglionic sympathetic neurons, the size of the dendritic arbor correlates with not only the number and pattern of synaptic inputs [3, 8], but also tonic activity [8, 9]. As is true of central neurons, aberrant morphology of sympathetic neuron dendrites is associated with disease. For example, dendritic hypertrophy of sympathetic neurons in stellate and superior cervical ganglia (SCG) is observed in the spontaneously hypertensive rat [10, 11], and is thought to contribute to the pathogenesis of hypertension in this model [11]. Therapeutic intervention with statins not only decreases sympathetic activity and normalizes blood pressure in the spontaneously hypertensive rat [12], but also decreases dendritic arborization of both stellate and SCG neurons [13].

In this chapter, we will review what is known about the molecular and cellular mechanisms that regulate dendrites in postganglionic sympathetic neurons, and identify key data gaps.

2. Early studies of dendritic growth in sympathetic neurons

The majority of dendritic growth in postganglionic sympathetic neurons occurs during the postnatal period; however, dendrites continue to grow into adulthood [14–16], and *in situ* imaging of mature sympathetic neurons has demonstrated that their dendritic arbors continually grow and retract throughout life [17, 18]. In the rat, neonatal deafferentation has negligible effect on dendritic growth in SCG neurons throughout the first month of life [19–21], indicating that dendritic growth in sympathetic neurons does not require afferent input. In contrast, target tissues strongly influence dendritic growth in these neurons. Experimentally reducing target size causes dendritic arbors of SCG neurons to be smaller than normal; conversely, increasing the target size significantly increases the size of the dendritic arbor [16]. The influence of target is further illustrated by observations that sympathetic neurons within the same ganglion that project to different targets exhibit varying dendritic morphologies [18, 19].

The effect of target tissues on dendritic growth in sympathetic neurons is mediated, at least in part, by nerve growth factor (NGF) [22–25]. Separation of neurons from target tissues by axonal ligation causes dendritic atrophy in the few neurons that survive, and this effect is attenuated by systemic administration of NGF [26, 27]. However, exogenous NGF reverses axotomy-induced dendritic retraction by <50%, even though cell survival is completely rescued [27], indicating that additional target-derived factors are needed to fully account for the effects of target on dendritic growth. Consistent with this conclusion, the dendritic complexity of axotomized sympathetic neurons recovers to control levels upon ganglion cell reinnervation of the periphery [28]. *In vitro* studies further suggest that factors in addition to NGF are

required for dendritic growth in sympathetic neurons. When grown in low-density cultures in the absence of serum or non-neuronal cells, but in the presence of optimal concentrations of NGF, sympathetic neurons form an axon but no dendrites [29, 30].

In vitro studies have further revealed that the initiation and maintenance of dendritic growth in sympathetic neurons is regulated by trophic interactions. Addition of serum to NGF-containing medium stimulates sympathetic neurons to form dendrites, although under these culture conditions, the dendritic arbor is significantly less complex than is observed *in vivo* [31]. In contrast, co-culture with ganglionic glial cells causes these neurons to form a dendritic arbor comparable to that of their *in vivo* counterparts [32]. Subsequently, it was discovered that the addition of bone morphogenetic proteins (BMPs) to the culture medium similarly triggers sympathetic neurons to form a complex dendritic arbor [29, 33]. Multiple BMP family members have been shown to stimulate dendritic growth in cultured sympathetic neurons, including BMPs 2, 4, 5, 6, 7 and 60A; however, this activity appears to be restricted to the dpp and 60A BMP subfamilies since BMP-3 and other members of the TGF β superfamily, including TGF β 1, TGF β 2, TGF β 3, activin A, inhibin, and GDNF, have no effect on dendritic growth in cultured sympathetic neurons [29, 33, 34].

The trophic actions of BMPs are specific to dendritic growth in that BMPs do not support cell survival, nor do they enhance axonal growth in cultured sympathetic neurons [29]. Consistent with observations of dendritic growth in sympathetic neurons *in vivo*, the dendrite-promoting activity of BMP-7 is independent of synaptic or electrical activity [35], but is modulated by NGF [29, 36]. Importantly, the dendritic arbor induced by BMPs in cultured sympathetic neurons is comparable to that of their *in vivo* counterparts with respect to not only size and complexity, but also accumulation and post-translational modification of dendrite-specific cytoskeletal and membrane proteins, exclusion of axonal proteins, transport of select mRNA, and formation of synaptic contacts of the appropriate polarity [29, 34, 35]. These observations indicate that BMPs selectively induce the execution of a developmental program in sympathetic neurons that controls both quantitative and qualitative aspects of dendritic growth.

These observations suggest that BMPs mediate the effects of ganglionic glia and target tissues on dendritic growth in sympathetic neurons. Immunocytochemical and *in situ* hybridization studies indicate that the spatiotemporal expression of BMPs 5, -6, and -7 in rat SCG is consistent with a role in the initial stages of dendritogenesis [33, 37]. *In vitro*, both SCG glia and neurons express BMP mRNA and protein when grown in the absence or presence of each other [37]. However, co-culture of sympathetic neurons with ganglionic glia markedly increases BMP protein coincident with a significant decrease in levels of the soluble BMP antagonists, follistatin and noggin [37]. Functional assays indicate that glial-induced dendritic growth is significantly reduced by BMP-7 antibodies and completely blocked by exogenous noggin and follistatin [37]. Collectively, these data suggest a model in which glia influence the rapid perinatal expansion of the dendritic arbor in sympathetic neurons by increasing BMP activity via modulation of the balance between BMPs and their antagonists. Whether this model holds true *in vivo* has yet to be tested.

The question of whether BMPs also contribute to target effects on dendritic growth has yet to be addressed experimentally. Sympathetic targets, including the eye, heart, lung, kidney, and

blood vessels, express significant levels of BMPs during embryonic development, throughout the postnatal period, and into adulthood [38–40]. Thus, target tissues may be a source of BMPs to sympathetic neurons not only during initial expansion of the dendritic arbor, but also in the maintenance and remodeling of dendritic arbors that continues throughout the life of the animal.

3. Signaling pathways that regulate dendritic growth in sympathetic neurons

Research over the past few decades has provided insights into the signaling pathways and molecular mechanisms that control dendritic growth in sympathetic neurons. As discussed in the preceding section, BMPs and NGF play predominant roles in the initiation and maintenance of dendrites in these autonomic neurons. While the importance of these growth factors as regulators of dendritic growth in sympathetic neurons is well established, the downstream effectors that link BMP and NGF to increased dendritic growth are not fully understood. In this section, we will discuss the signaling pathways activated by these growth factors, the evidence implicating downstream effectors of BMPs and NGF in dendritic regulation, and the identification of factors that interact with these signaling pathways to alter their influence on the dendritic arborization of sympathetic neurons.

3.1. BMP signaling

BMPs mediate their cellular effects by binding to a heteromeric receptor complex of transmembrane serine/threonine kinase receptor subunits comprised of a type I receptor [BMP type I receptor A (BMPRI A), which is also known as activin receptor-like kinase-3 (ALK-3) or BMP type I receptor B (BMPRI B, also known as ALK-6)], and a type II receptor [BMP type II receptor (BMPRII), activin type II receptor (ActRII), or activin type IIB receptor (ActRIIB)] [41, 42]. Ligand binding causes type II receptors to phosphorylate type I receptors, which then phosphorylate Smads 1, 5, and/or 8, also known as receptor Smads (R-Smads). Phosphorylated R-Smads complex with Smad 4, triggering translocation of the Smad complex to the nucleus to regulate gene transcription [42, 43]. Studies in *Smad* knockout animals and *Smad* deficient cells suggest that BMPs can also signal through Smad-independent pathways via activation of mitogen-activated protein kinase (MAPK), c-jun amino terminal kinase (JNK), p38-MAPK, phosphoinositol-3-kinase (PI3K), LIM kinase 1 (LIMK1) or small GTPases [43–46].

BMP signaling pathways are active in sympathetic ganglia during developmental periods corresponding to the initiation, extension and maintenance of dendrites. Quantitative PCR, *in situ* hybridization, and immunocytochemistry studies have confirmed the presence of mRNA and protein for BMPRI A, BMPRI B, ActRII and BMPRII in neurons of mouse SCG starting as early as embryonic day 14, and persisting into postnatal development and even adulthood [47, 48]. A role for BMPRI A in regulating dendritic growth in sympathetic neurons *in vivo* was confirmed in a BMPRI A conditional knockout mouse generated by crossing a *Dbhi-Cre* mouse line to a *Bmpr1a* floxed line [49]. *Dbhi-Cre* is expressed in sympathetic ganglia around E10.5, thus, *Bmpr1a* expression is knocked out after neuronal specification, but before initiation of dendritic growth. The loss of BMPRI A expression in sympathetic ganglia significantly

diminished but did not completely block dendritic growth *in vivo* [49]. At postnatal day 3, the number of primary dendrites and the size of the dendritic arbor were not significantly different in sympathetic neurons of SCG from *Bmpr1a* conditional knockout mice *vs.* SCG neurons from congenic wildtype controls. At later developmental times, however, dendritic arborization was significantly decreased in SCG neurons of *Bmpr1a* knockout *vs.* wildtype mice. These data suggest that, *in vivo*, BMP signaling is required for the maintenance of dendrites, but not for the initiation and early growth of dendrites in sympathetic neurons.

Several caveats of the *in vivo* study suggest that the lack of effect of *Bmpr1a* knockout on early stages of dendritic growth *in vivo* may not be discrepant with *in vitro* data showing that BMPs are necessary and sufficient for induction of dendritic growth in sympathetic neurons [29, 49]. First, while the authors confirmed that *Bmpr1a* mRNA was not generated in the SCG after embryonic day 11.5, they did not examine BMPRIA protein levels in the SCG of knockout animals. Thus, the possibility that BMPRIA protein was still present during early stages of dendritic growth cannot be ruled out. Moreover, recent biochemical studies have shown that BMPRs cycle between various cellular compartments, and the association of the receptors with endocytosis machinery is required for Smad-mediated signal transduction [50]. Thus, additional studies are needed to characterize BMPR turnover and changes in receptor localization in wildtype *vs.* BMPR knockouts. Another, perhaps more likely, explanation is that sympathetic neurons in the *Bmpr1a* knockout mice express other type I receptors, such as the activin receptors, that may be activated by BMPs to trigger dendritic growth at early developmental stages. Further studies targeting other BMPRs are warranted to fully elucidate the role of BMP signaling in dendritogenesis *in vivo*.

3.1.1. Smad-dependent transcriptional regulation of dendritic growth

Canonical BMP signaling involves the Smad family of transcription factors. Immunocytochemical analyses of primary rat SCG neurons have demonstrated that Smad 1/5/8 translocates to the nucleus within 20 minutes of exposure to BMP-7, with maximal nuclear translocation observed within 2 hours of adding BMP-7 to the culture medium. Transfection with a Smad1 dominant negative mutant, Smad1 (3SA), blocked BMP-7-induced dendritic growth in primary sympathetic neurons [51], indicating that Smad 1 activation is necessary for induction of dendritic growth by BMP-7. In contrast to the *Bmpr1a* conditional knockouts, conditional knockdown of *Smad4* in sympathetic neurons, generated by crossing *Dbhi-Cre* mice to *Smad4* floxed mice, increased dendritic length and the size of the dendritic arbor in SCG neurons [49], suggesting that Smad 4 may play a role in limiting dendritic growth *in vivo*.

A comprehensive analyses of Smad-dependent dendritic growth in sympathetic neurons provided evidence for early transcriptional regulation of dendritic growth downstream of BMP-7 [52]. In neuronal cell cultures from embryonic rat SCG, BMP-7-induced dendritic growth could be blocked by pharmacologic inhibition of transcription with actinomycin-D when the inhibitor was added within the first 24 hours of BMP-7 exposure but not when it was added after 48 hours of BMP-7 exposure. Microarray analyses identified over 250 genes that were differentially regulated by BMP-7 within the first 24 hours after adding BMP-7 to the culture medium. Of these, 56 mRNAs were altered within the first 6 hours and 185 mRNAs were differentially regulated at 24 hours after BMP exposure [52]. Many of the differentially regulated genes were linked to signaling pathways previously implicated in dendritogenesis in other

neuronal cell types or neuronal morphogenesis and/or axonal guidance, such as BMP, Notch, integrin, Wnt, and NGF signaling molecules. However, the functional relevance of most of these genes to dendritic growth in sympathetic neurons has yet to be determined. Moreover, recent reports of limited correlation between transcriptome and proteome analysis in yeast, plants and mice [53, 54], suggest that in order to generate a more complete understanding of the molecular pathways that link BMPs to dendritic growth in sympathetic neurons, detailed proteome analyses are needed to complement the existing transcriptomic dataset.

One gene identified as being strongly upregulated by BMP-7 in primary sympathetic neurons, the gene encoding the p75 neurotrophin receptor (p75^{NTR}) [52], has been evaluated for a role in BMP-induced dendritic growth. A member of the tumor necrosis factor (TNF) receptor family, p75^{NTR} regulates diverse neurobiological processes, including axonal growth, synaptic plasticity, dendritic growth in central neurons, and neuronal cell death [55–61]. p75^{NTR} binds diverse ligands to mediate its effects, including NGF, other neurotrophins, myelin-derived polypeptides, such as myelin-associated glycoprotein (MAG) or Nogo, and β -amyloid peptide [60, 62, 63]. In cultured embryonic rat SCG neurons, p75^{NTR} mRNA and protein expression are significantly upregulated within 24 hours of exposure to BMP-7 [52, 64], and pharmacologic inhibition of signaling via BMPRI prevents induction of p75^{NTR} protein expression in primary sympathetic neurons exposed to BMP-7 [64]. Functional studies revealed that BMP7 does not trigger dendritic growth in primary sympathetic neurons derived from SCG of p75^{NTR} knockout mice; conversely, ligand-independent activation of p75^{NTR} via overexpression of a p75^{NTR} cDNA construct in p75^{NTR}^{-/-} neurons [65], phenocopies the dendrite-promoting effects of BMP-7 [64]. Morphometric analyses of SCG from wildtype *vs.* p75^{NTR} knockout mice at 3, 6 and 12–16 weeks of age indicated that genetic deletion of p75^{NTR} does not prevent dendritic growth, but does significantly stunt dendritic maturation in sympathetic neurons. These data support the hypotheses that p75^{NTR} is involved in downstream signaling events that mediate BMP7-induced dendritic growth in sympathetic neurons, and suggest that p75^{NTR} signaling positively modulates dendritic complexity in sympathetic neurons *in vivo*.

An outstanding question regarding p75^{NTR} effects on dendritic growth in sympathetic neurons is the identity of ligand(s) and co-receptor(s) that p75^{NTR} interacts with to mediate BMP-induced dendritic growth. Several lines of evidence argue against a direct interaction between p75^{NTR} and the BMP receptor complex: (i) inhibiting transcription blocks both BMP-induced dendritic growth and upregulation of p75^{NTR} mRNA [52]; (ii) pharmacologic blockade of BMPRI signaling blocks p75^{NTR} induction [64]; and (iii) overexpressing p75^{NTR} in cultured p75^{NTR}^{-/-} neurons induces dendritic growth even in the absence of BMP-7 [64]. NGF is a potential activating ligand, and NGF is required for BMP-induced dendritic growth [29, 36]. However, the observation that p75^{NTR} interactions with the primary receptor for NGF, TrkA, serve to limit synapse formation in sympathetic neurons [66], argues against p75^{NTR}-TrkA interactions enhancing dendritic development. Likewise, pro-neurotrophin activation of the p75^{NTR}-sortilin complex serves to promote sympathetic axon degeneration and cell death [67], suggesting that this interaction is also unlikely to stimulate dendritic growth. The identity of the p75^{NTR} ligand that mediates BMP-7-induced dendritic growth remains an outstanding question.

Similarly, the downstream effector molecule(s) that link p75^{NTR} to increased dendritic arborization remain to be determined. Key candidates include the Rho GTPases. Rho GTPases function as central regulators of dendritic morphology, linking extracellular signals to changes

in the dendritic actin cytoskeleton [68, 69]. p75^{NTR} has been shown to interact with RhoA in the yeast two-hybrid system [70]. In cultured rat sympathetic neurons, exposure to BMP-7 increases the levels of the GTP-bound form of RhoA, but not GTP-Rac1 or GTP-Cdc42, as determined by a GTP pull down assay, and triggers RhoA translocation from the cytoplasm to the membrane [13]. The observation that BMP-7-induced dendritic growth in primary sympathetic neurons requires RhoA activation [13], suggests a model in which BMP-7 sequentially activates BMPRIA, Smad 1/5/8, p75^{NTR}, and then RhoA to induce dendritic growth in sympathetic neurons.

3.2. Signaling pathways that interact with Smad signaling to modulate BMP-induced dendritic growth

The shape of the dendritic arbor of developing sympathetic neurons is determined by interactions between positive and negative regulators of dendritic growth. A number of signaling pathways have been shown to interact with BMP signaling to modulate the number of dendrites, total dendritic length and dendritic branching in sympathetic neurons. In this section, we will review known positive and negative regulators of Smad signaling that impact BMP-induced dendritogenesis in sympathetic neurons.

3.2.1. Positive regulators of Smad signaling that enhance dendritic growth

Proteasome-mediated signaling. The ubiquitin-proteasome pathway is unique in that it is one of the few signaling pathways identified thus far whose interactions with BMP signaling enhance dendritic arborization in sympathetic neurons. In the nervous system, proteasome-mediated protein degradation pathways are important for self-renewal of neurons, axonal growth, vesicle release, receptor turnover, signal transduction and synaptic plasticity [71–73]. The ubiquitin-proteasome pathway has been implicated in regulating dendritic growth in the mammalian central nervous system, with multiple E3 ubiquitin ligases, including Anaphase promoting complex (APC), cullin RING-type E3 ubiquitin ligases (CUL) and neuronal precursor cell expressed and developmentally downregulated protein (Nedd4), determined to be necessary for the extension and maintenance of the dendritic arbor of central neurons [71, 74–76].

Biochemical studies have shown that R-Smads interact with components of the proteasome complex, as well as enzymes and proteins involved in the ubiquitination-deubiquitination of proteins, in many systems, and the ability of Smads to regulate transcription is dependent on association with the proteasome complex in various cell types [77, 78]. Interactions between BMP signaling molecules and the proteasome pathway have been reported in perinatal rat sympathetic neuronal cultures prior to dendritic growth induction by BMP-7 [51]. In this study, interactions between Smad1 and multiple proteasome components were confirmed using a yeast two-hybrid assay, and pharmacologic inhibition of proteasome activity by lactacystin and ALLN (N-acetyl-Leu-Leu-norleucinal) selectively blocked BMP-7-induced dendritic growth in primary sympathetic neurons in the absence of any effect on axonal growth [51]. These proteasome inhibitors also suppressed Smad-mediated transcriptional regulation in a biochemical assay using a Tlx-luciferase construct transfected into P19 cells [51]. One caveat of this study is that although there was clearly a functional interaction between BMP signaling and the proteasome pathway in the context of dendritic growth, a biochemical

interaction between Smads and proteasomes were not demonstrated in primary sympathetic neurons. Further studies are necessary to fully understand the genetic and biochemical interactions between Smads and ubiquitin-proteasome pathway during dendritogenesis in sympathetic neurons.

Signaling mediated by reactive oxygen species (ROS). Under physiological conditions, ROS are generated by the mitochondrial electron transport chain or as a consequence of the activity of NADPH oxidase (NOX) [79–81]. While the deleterious effects of ROS at high concentrations are well documented, over the past decade, there is growing appreciation of the beneficial role of ROS at physiological concentrations [80–84], and of the importance of the NOX family of enzymes in regulating many aspects of neuronal development including neurogenesis, neurite outgrowth and synaptic plasticity [80, 83, 85]. NOX2 is expressed in neonatal rat SCG neuronal cell cultures [86], and exposure of cultured embryonic SCG neurons to BMP-7 increases the expression of NOX2 [87]. Pharmacologic inhibition or siRNA knockdown of NOX2 significantly decreases BMP-7-induced dendritic growth in primary sympathetic neurons, an effect that is also observed in cultures co-exposed to BMP-7 and any of three mechanistically and structurally distinct antioxidants that block ROS generation [87]. Antioxidants block BMP-induced dendritic growth downstream of Smad signaling since BMP-induced nuclear translocation of Smads is unaffected by antioxidant treatment [87].

Collectively, these data support the hypothesis that ROS are involved in the downstream signaling events that mediate BMP7-induced dendritic growth, and suggest that ROS-mediated signaling positively modulates dendritic complexity in sympathetic neurons. One caveat of this study, however, is that while BMP-7 was observed to increase NOX2 levels and oxygen consumption in sympathetic neurons, increased ROS levels were not detected in sympathetic neurons exposed to BMP-7. Likely, this reflects the fact that physiologic BMP signaling generates levels of ROS that are below the detection threshold for standard ROS detection assays. Further work is needed to determine whether these *in vitro* observations translate to a role for ROS in regulating dendritic growth in sympathetic ganglia *in vivo*.

3.2.2. Negative regulators of SMAD signaling that influence dendritic growth

STAT signaling. The interferons (IFN) and the neuropoietic cytokines, which include interleukins (IL), leukemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF), signal through the activation and nuclear translocation of signal transducers and activators of transcription (STAT) proteins, which then regulate transcription of target genes [88]. Previous studies have shown that cytokines that signal through STATs *vs.* members of the TGF β superfamily of signaling molecules tend to exert opposing effects on cellular function [89–91]. Similar antagonistic interactions between STAT-dependent cytokine signaling pathways and the BMP signaling pathways have been documented during dendritogenesis in perinatal sympathetic neurons. In primary perinatal sympathetic neurons, addition of LIF, CNTF or gamma-IFN (IFN γ) significantly decreased the number of primary dendrites and total dendritic length in cultures exposed to BMP-7, but had no effect on axonal growth or cell viability [92–94]. In addition, all three cytokines triggered retraction of preexisting dendrites [92, 94], suggesting a role for them in the dendritic retraction observed following neuronal injury. Addition of LIF, CNTF or IFN γ to primary sympathetic neurons resulted in nuclear translocation of STAT, and transfection of a dominant negative STAT1 construct blocked

IFN γ -mediated dendritic retraction [92, 94]. These data provide evidence that neuropoietic cytokines negatively regulate dendritic growth in perinatal sympathetic neurons via activation of STAT proteins. However, many questions remain, including the mechanism by which nuclear translocation of STAT proteins limits the activity of SMAD proteins, and the downstream targets of STAT and SMAD signaling during dendritogenesis.

Mitogen-activated protein kinase (MAPK) signaling. MAPK family members, including extracellular signal-regulated kinase (ERK), p38, and c-Jun NH $_2$ -terminal kinase (JNK), are serine–threonine kinases that function as downstream effectors of many extracellular signaling molecules, including NGF, epidermal growth factor (EGF), and fibroblast growth factor (FGF) [95, 96]. The activation of MAPK regulates gene expression to alter various cellular activities such as cell division, growth, survival and cell death [95, 96]. Previous studies in *Xenopus* embryos and cells such as radial glia, astrocytes, hippocampal neurons, and PC12 cells, have demonstrated interplay between Smad and MAPK signaling pathways, and shown that phosphorylation of the linker on the Smad protein by MAPK is necessary for coordinating the cellular effects of BMPs and other growth factors [43, 97–99]. Interplay between BMP and MAPK signaling pathways is also observed during dendritogenesis in rat and mouse sympathetic neurons. Pharmacologic inhibition of MAPK signaling with PD98059 or transfection of a dominant negative MEK1 or ERK2 mutant increases the number of dendrites and total dendritic arbor induced following BMP-7 exposure, whereas overexpression of MEK1 decreases BMP-7 induced dendritic growth [100]. Inhibition of MAPK signaling in primary sympathetic neurons also increases Smad nuclear accumulation following BMP-7 exposure, and FGF was identified as one of the ligands responsible for activating the ERK signaling pathway in these neurons [100]. These findings suggest that, in perinatal sympathetic neurons, FGF activates the MAPK pathway, which inhibits the nuclear accumulation of SMADs, thereby limiting BMP-induced dendritic growth.

In addition to modulating BMP effects on dendritic arborization via activation of MAPK signaling, FGFs regulate neuronal differentiation via the integrative nuclear FGFR1 signaling (INFS) pathway [101]. FGFR1 is expressed in the nucleus of adult rat SCG neurons following axotomy [102]. Nuclear localization of FGFR1 is also increased in perinatal rat sympathetic neurons following exposure to BMP-7, and transfection of a mutant FGFR1 receptor inhibits FGFR1 nuclear localization and decreases the dendritic response to BMP-7 [103]. These data suggest that the INFS-mediated FGF signaling pathway functions downstream of BMP signaling to limit BMP-induced dendritogenesis in sympathetic neurons.

Rit GTPase, a member of the small GTPase family, has also been shown to activate ERK1/2 in primary sympathetic neurons, and the transfection of dominant negative (dn) Rit or constitutively active (ca) Rit were observed to increase or decrease BMP-induced dendritic growth, respectively [36]. Rit GTPase also negatively modulates dendritic growth as a downstream target of IFN γ signaling as demonstrated by inhibition of IFN γ -mediated dendritic retraction in primary sympathetic neurons transfected with dnRit constructs [104]. Addition of IFN γ to cultures of pheochromocytoma cells, which are often used as a model for sympathetic neurons, increased levels of GTP-Rit, and transfection of dnRit inhibited IFN γ -induced activation of p38 MAPK [104]. These observations suggest that a novel Rit-p38 MAP kinase signaling pathway functions in parallel with the canonical JAK–STAT signaling pathway to mediate IFN γ -induced dendritic retraction. Collectively, these studies provide evidence for crosstalk

between BMP signaling and MAPK signaling during dendritogenesis in sympathetic neurons, and suggest that in contrast to its effects in central neurons, MAPK signaling functions as a negative regulator of BMP-induced dendritic growth in sympathetic neurons.

Retinoic acid signaling. In the developing nervous system, BMPs and retinoic acid work synergistically to regulate neural tube patterning and specification of neuronal identity [105–108]. Similarly, interplay between BMP and retinoic acid signaling have been documented during dendritogenesis in sympathetic neurons; however, their interaction in this latter context is antagonistic [106]. Genes required for synthesis of retinoic acid are expressed in cultured perinatal rat SCG neurons, and retinoids synthesized by SCG explants were able to activate transcriptional reporters in retinoic acid-responsive F9 cells [106]. The addition of retinoic acid, or specific retinoic acid receptor and retinoid X receptor agonists, to the medium of cultured sympathetic neurons inhibited BMP-induced dendritic growth without altering axonal growth or cell viability [106]. What is not known, however, is the point of convergence between these two pathways in the context of dendritic growth. Further studies are also needed to determine whether retinoic acid regulates the dendritic arborization of sympathetic neurons *in vivo*.

Signaling by pituitary adenylate cyclase (PACAP) and vasoactive intestinal peptide (VIP). PACAP is a member of the secretin/VIP family of peptides that regulates the development of cells within the sympathoadrenal lineage [109]. In sympathetic neurons, PACAP regulates cell survival, proliferation, and catecholamine secretion, and release of PACAP and VIP from preganglionic neurons stimulates depolarization of sympathetic neurons [109]. In cultured perinatal rat sympathetic neurons, PACAP38 and VIP decrease BMP-induced dendritic growth, as evidenced by a decrease in the percentage of cells with dendrites, number of dendrites per neuron, and size of the dendritic arbor [110]. Using receptor specific antagonists and antibodies against phosphorylated cyclic AMP response element binding (CREB) protein, the PACAP response was shown to be mediated by PAC₁ receptor activation, which results in the nuclear accumulation of phospho-CREB. Inhibition of adenylate cyclase activity by SQ22526 overcomes the inhibitory effects of PACAP on dendritic growth, and agents that increase cAMP levels, such as forskolin, inhibit BMP-induced dendritic growth [110]. These data suggest that peptides released by preganglionic nerves modulate dendritic growth in postganglionic sympathetic neurons by a cAMP-dependent mechanism.

In summary, signaling by cytokines, growth factors, small molecules, and peptides, such as retinoic acid, PACAP and VIP, antagonize BMP signaling during dendritogenesis in sympathetic neurons. Most of the relevant data were collected from studies of primary perinatal sympathetic neurons cultured from rodent SCG. While the findings from this model have provided a glimpse into the complexity of the interactions that influence dendritic arborization of these neurons, further studies are required to understand the mechanisms by which these factors interact to regulate dendritic growth, how these pathways are spatially and temporally coordinated to influence dendritic arborization of sympathetic neurons *in vivo*, and to determine their relevance to the human condition.

3.3. Other pathways that regulate dendritic growth in sympathetic neurons

As described earlier, NGF is an important regulator of dendritic growth in sympathetic neurons. However, the molecular mechanisms by which NGF regulates dendritic growth are

not well characterized. Early growth response-3 (*Egr3*), a transcriptional regulator known to be induced by NGF via MAPK signaling, has been identified as a potential downstream regulator of NGF-induced dendritic growth [111]. Sympathetic neurons from a conditional *Egr3* knockout generated using *Dbhi-Cre* have fewer primary dendrites and shorter dendritic arbors compared to sympathetic neurons from congenic wildtype animals. In addition, *Egr3* knockout animals exhibit defects in axonal guidance, and in innervation of autonomic targets [111]. Although, *Egr3* had previously been shown to not be required for NGF effects on neuronal survival [112], some neuronal cell death was observed in germline mutants for *Egr3*. It is, therefore, possible that the dendritic effects of knocking out *Egr3* are secondary to adverse effects on neuronal cell viability or changes in target innervation.

Neuronal depolarization induced by electric field stimulation or the addition of potassium chloride to primary postnatal sympathetic neurons cultured was shown to trigger the formation of dendrites in the presence of NGF that retracted in the absence of neuronal activity [113]. Neuronal depolarization enhanced stability of microtubules and activated calcium calmodulin dependent kinase II (CaMKII) in dendrites. The latter was shown to be causally related to the effects of neuronal depolarization on dendritic growth: pharmacologic inhibition of CaMKII activity using KN62 or mAIP completely blocks activity-dependent dendritic growth in cultured sympathetic neurons [113].

Signaling by integrin-linked kinase (ILK) and glycogen synthase kinase-3 β (GSK-3 β) have also been shown to be downstream effectors of activity-dependent dendritic growth in postnatal sympathetic neurons [114]. ILK and GSK-3 β are serine threonine kinases that are downstream effectors of integrin and neurotrophin signaling [115]. ILK has been shown to phosphorylate and inactivate GSK-3 β to regulate NGF-mediated axonal growth [116]. Increased phosphorylation of GSK-3 β protein was observed in cultured postnatal rat SCG neurons in response to increased neuronal activity, and inhibition of ILK activity by QLT0254, as well as transfection of dominant negative ILK or siRNA for ILK, blocked activity-dependent dendritic growth in these neurons. Similarly, inhibition of GSK-3 β activity using kenpaullone or genetic knock-down of GSK-3 β expression increased the number of primary dendrites formed in response to potassium chloride, suggesting that GSK-3 β inhibition is necessary for early stages of activity-dependent dendritic growth in sympathetic neurons.

Interestingly, unlike BMP-induced dendritic growth, inhibition of ERK activity inhibited activity-dependent dendritic growth in postnatal sympathetic neurons *in vitro* [113, 114]. Pharmacologic inhibition of ERK by PD98059 blocked activity-dependent dendritic growth, inhibition of GSK-3 β increased depolarization-dependent ERK activation, and inhibition of ERK reversed the enhanced dendritic growth observed with GSK-3 β inhibition [113, 114]. The reasons for the opposing roles of ERK in BMP-induced vs. activity-dependent dendritic growth remain unexplored.

4. The path forward

The experimental evidence clearly implicate NGF, BMP and neuronal activity as positive regulators of dendritic growth in perinatal sympathetic neurons *in vitro*. However, the interaction between these three pathways and their relative contributions to the induction and

maintenance of dendritic growth in sympathetic neurons *in vivo* are not fully understood. Multiple mechanistically and structural diverse signaling molecules have been implicated as negative regulators of dendritic growth *in vitro*. The spatial and temporal regulation of these pathways, how they interact with each other and with positive modulators of dendritic growth to shape dendritic arbors, and their functional significance *in vivo* remain outstanding questions. In addition, further studies are needed to understand the genes and proteins that are regulated downstream of each of these signaling pathways and to identify those that serve as hubs for interactions between multiple signaling pathways to regulate the final dendritic arbor of sympathetic neurons. Finally, a key data gap is the relevance of these observations in experimental models to the human condition.

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Conflict of interest

The authors declare no conflict of interest.

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References

- [1] Miller JP, Jacobs GA. Relationships between neuronal structure and function. *The Journal of Experimental Biology*. 1984;**112**:129-145
- [2] Purpura DP. Comparative physiology of dendrites. In: Quarten GC, Melnechuk T, Schmitt FO, editors. *The Neurosciences: A Study Program*. New York: Rockefeller University Press; 1967. pp. 372-393

- [3] Purves D. *Body and Brain: A Trophic Theory of Neural Connections*. Cambridge, MA: Harvard University Press; 1988
- [4] Kweon JH, Kim S, Lee SB. The cellular basis of dendrite pathology in neurodegenerative diseases. *BMB Reports*. 2017;**50**(1):5-11
- [5] Copf T. Impairments in dendrite morphogenesis as etiology for neurodevelopmental disorders and implications for therapeutic treatments. *Neuroscience and Biobehavioral Reviews*. 2016;**68**:946-978. DOI: 10.1016/j.neubiorev.2016.04.008
- [6] Lein PJ, Fryer AD, Higgins D. Cell culture: Autonomic and enteric neurons. In: Squire LR, editor. *Encyclopedia of Neuroscience*. Oxford, UK: Academic Press; 2009. pp. 625-632
- [7] Purves D, Lichtman JW. Geometrical differences among homologous neurons in mammals. *Science*. 1985;**228**(4697):298-302
- [8] Ivanov A, Purves D. Ongoing electrical activity of superior cervical ganglion cells in mammals of different size. *The Journal of Comparative Neurology*. 1989;**284**(3):398-404
- [9] De Castro F, Sanchez-Vives MV, Munoz-Martinez EJ, Gallego R. Effects of postganglionic nerve section on synaptic transmission in the superior cervical ganglion of the guinea-pig. *Neuroscience*. 1995;**67**(3):689-695
- [10] Peruzzi D, Hendley ED, Forehand CJ. Hypertrophy of stellate ganglion cells in hypertensive, but not hyperactive, rats. *The American Journal of Physiology*. 1991;**261**(4 Pt 2):R979-R984
- [11] Kondo M, Terada M, Shimizu D, Fujiwara T, Tabei R. Morphometric study of the superior cervical and stellate ganglia of spontaneously hypertensive rats during the prehypertensive stage. *Virchows Archiv. B, Cell Pathology Including Molecular Pathology*. 1990;**58**(5):371-376
- [12] Kishi T, Hirooka Y, Mukai Y, Shimokawa H, Takeshita A. Atorvastatin causes depressor and sympatho-inhibitory effects with upregulation of nitric oxide synthases in stroke-prone spontaneously hypertensive rats. *Journal of Hypertension*. 2003;**21**(2):379-386
- [13] Kim WY, Gonsiorek EA, Barnhart C, Davare MA, Engebose AJ, Lauridsen H, Bruun D, Lesiak A, Wayman G, Bucelli R, Higgins D, Lein PJ. Statins decrease dendritic arborization in rat sympathetic neurons by blocking RhoA activation. *Journal of Neurochemistry*. 2009;**108**(4):1057-1071
- [14] Andrews TJ, Li D, Halliwell J, Cowen T. The effect of age on dendrites in the rat superior cervical ganglion. *Journal of Anatomy*. 1994;**184**(Pt 1):111-117
- [15] Snider WD. Rostrocaudal differences in dendritic growth and synaptogenesis in rat sympathetic chain ganglia. *The Journal of Comparative Neurology*. 1986;**244**(2):245-253. DOI: 10.1002/cne.902440210
- [16] Voyvodic JT. Peripheral target regulation of dendritic geometry in the rat superior cervical ganglion. *The Journal of Neuroscience*. 1989;**9**(6):1997-2010

- [17] Purves D, Hadley RD, Voyvodic JT. Dynamic changes in the dendritic geometry of individual neurons visualized over periods of up to three months in the superior cervical ganglion of living mice. *The Journal of Neuroscience*. 1986;**6**(4):1051-1060
- [18] Andrews TJ, Thrassivoulou C, Nesbit W, Cowen T. Target-specific differences in the dendritic morphology and neuropeptide content of neurons in the rat SCG during development and aging. *The Journal of Comparative Neurology*. 1996;**368**(1):33-44
- [19] Luebke JI, Wright LL. Characterization of superior cervical ganglion neurons that project to the submandibular glands, the eyes, and the pineal gland in rats. *Brain Research*. 1992;**589**(1):1-14
- [20] Smolen AJ, Beaston-Wimmer P. Dendritic development in the rat superior cervical ganglion. *Brain Research*. 1986;**394**(2):245-252
- [21] Voyvodic JT. Development and regulation of dendrites in the rat superior cervical ganglion. *The Journal of Neuroscience*. 1987;**7**(3):904-912
- [22] Goedert M, Otten U, Thoenen H. Biochemical effects of antibodies against nerve growth factor on developing and differentiated sympathetic ganglia. *Brain Research*. 1978;**148**(1):264-268
- [23] Purves D, Snider WD, Voyvodic JT. Trophic regulation of nerve cell morphology and innervation in the autonomic nervous system. *Nature*. 1988;**336**(6195):123-128
- [24] Ruit KG, Osborne PA, Schmidt RE, Johnson EM Jr, Snider WD. Nerve growth factor regulates sympathetic ganglion cell morphology and survival in the adult mouse. *The Journal of Neuroscience*. 1990;**10**(7):2412-2419
- [25] Ruit KG, Snider WD. Administration or deprivation of nerve growth factor during development permanently alters neuronal geometry. *The Journal of Comparative Neurology*. 1991;**314**(1):106-113
- [26] Purves D, Nja A. Effect of nerve growth factor on synaptic depression after axotomy. *Nature*. 1976;**260**(5551):535-536
- [27] Nja A, Purves D. The effects of nerve growth factor and its antiserum on synapses in the superior cervical ganglion of the guinea-pig. *The Journal of Physiology*. 1978;**277**:55-75
- [28] Yawo H. Changes in the dendritic geometry of mouse superior cervical ganglion cells following postganglionic axotomy. *The Journal of Neuroscience*. 1987;**7**(11):3703-3711
- [29] Lein P, Johnson M, Guo X, Rueger D, Higgins D. Osteogenic protein-1 induces dendritic growth in rat sympathetic neurons. *Neuron*. 1995;**15**(3):597-605
- [30] Bruckenstein DA, Higgins D. Morphological differentiation of embryonic rat sympathetic neurons in tissue culture. I. Conditions under which neurons form axons but not dendrites. *Developmental Biology*. 1988;**128**(2):324-336
- [31] Bruckenstein DA, Higgins D. Morphological differentiation of embryonic rat sympathetic neurons in tissue culture. II. Serum promotes dendritic growth. *Developmental Biology*. 1988;**128**(2):337-348. DOI: 10.1016/0012-1606(88)90296-5

- [32] Tropea M, Johnson MI, Higgins D. Glial cells promote dendritic development in rat sympathetic neurons in vitro. *Glia*. 1988;**1**(6):380-392
- [33] Beck HN, Drahushuk K, Jacoby DB, Higgins D, Lein PJ. Bone morphogenetic protein-5 (bmp-5) promotes dendritic growth in cultured sympathetic neurons. *BMC Neuroscience*. 2001;**2**:12
- [34] Guo X, Rueger D, Higgins D. Osteogenic protein-1 and related bone morphogenetic proteins regulate dendritic growth and the expression of microtubule-associated protein-2 in rat sympathetic neurons. *Neuroscience Letters*. 1998;**245**(3):131-134
- [35] Lein P, Guo X, Hedges AM, Rueger D, Johnson M, Higgins D. The effects of extracellular matrix and osteogenic protein-1 on the morphological differentiation of rat sympathetic neurons. *International Journal of Developmental Neuroscience*. 1996;**14**(3):203-215
- [36] Lein PJ, Guo X, Shi GX, Moholt-Siebert M, Bruun D, Andres DA. The novel GTPase Rit differentially regulates axonal and dendritic growth. *The Journal of Neuroscience*. 2007;**27**(17):4725-4736. DOI: 10.1523/JNEUROSCI.5633-06.2007
- [37] Lein PJ, Beck HN, Chandrasekaran V, Gallagher PJ, Chen HL, Lin Y, Guo X, Kaplan PL, Tiedge H, Higgins D. Glia induce dendritic growth in cultured sympathetic neurons by modulating the balance between bone morphogenetic proteins (BMPs) and BMP antagonists. *The Journal of Neuroscience*. 2002;**22**(23):10377-10387
- [38] Lyons KM, Hogan BL, Robertson EJ. Colocalization of BMP 7 and BMP 2 RNAs suggests that these factors cooperatively mediate tissue interactions during murine development. *Mechanisms of Development*. 1995;**50**(1):71-83
- [39] Vukicevic S, Latin V, Chen P, Batorsky R, Reddi AH, Sampath TK. Localization of osteogenic protein-1 (bone morphogenetic protein-7) during human embryonic development: High affinity binding to basement membranes. *Biochemical and Biophysical Research Communications*. 1994;**198**(2):693-700
- [40] Wall NA, Blessing M, Wright CV, Hogan BL. Biosynthesis and in vivo localization of the decapentaplegic-Vg-related protein, DVR-6 (bone morphogenetic protein-6). *The Journal of Cell Biology*. 1993;**120**(2):493-502
- [41] Katagiri T, Watabe T. Bone morphogenetic proteins. *Cold Spring Harbor Perspectives in Biology*. 2016;**8**(6). DOI: 10.1101/cshperspect.a021899
- [42] Massague J, Wotton D. Transcriptional control by the TGF-beta/Smad signaling system. *The EMBO Journal*. 2000;**19**(8):1745-1754. DOI: 10.1093/emboj/19.8.1745
- [43] Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature*. 2003;**425**(6958):577-584
- [44] Lee-Hoeflich ST, Causing CG, Podkowa M, Zhao X, Wrana JL, Attisano L. Activation of LIMK1 by binding to the BMP receptor, BMPRII, regulates BMP-dependent dendritogenesis. *The EMBO Journal*. 2004;**23**(24):4792-4801

- [45] Xu X, Han J, Ito Y, Bringas P Jr, Deng C, Chai Y. Ectodermal Smad4 and p38 MAPK are functionally redundant in mediating TGF-beta/BMP signaling during tooth and palate development. *Developmental Cell*. 2008;**15**(2):322-329. DOI: 10.1016/j.devcel.2008.06.004
- [46] Yu L, Hebert MC, Zhang YE. TGF-beta receptor-activated p38 MAP kinase mediates Smad-independent TGF-beta responses. *The EMBO Journal*. 2002;**21**(14):3749-3759. DOI: 10.1093/emboj/cdf366
- [47] O'Keeffe GW, Gutierrez H, Howard L, Laurie CW, Osorio C, Gavalda N, Wyatt SL, Davies AM. Region-specific role of growth differentiation factor-5 in the establishment of sympathetic innervation. *Neural Development*. 2016;**11**:4. DOI: 10.1186/s13064-016-0060-3
- [48] Zhang D, Mehler MF, Song Q, Kessler JA. Development of bone morphogenetic protein receptors in the nervous system and possible roles in regulating trkC expression. *The Journal of Neuroscience*. 1998;**18**(9):3314-3326
- [49] Majdazari A, Stubbusch J, Muller CM, Hennchen M, Weber M, Deng CX, Mishina Y, Schutz G, Deller T, Rohrer H. Dendrite complexity of sympathetic neurons is controlled during postnatal development by BMP signaling. *The Journal of Neuroscience*. 2013;**33**(38):15132-15144. DOI: 10.1523/JNEUROSCI.4748-12.2013
- [50] Ehrlich M. Endocytosis and trafficking of BMP receptors: Regulatory mechanisms for fine-tuning the signaling response in different cellular contexts. *Cytokine & Growth Factor Reviews*. 2016;**27**:35-42. DOI: 10.1016/j.cytogfr.2015.12.008
- [51] Guo X, Lin Y, Horbinski C, Drahushuk KM, Kim IJ, Kaplan PL, Lein P, Wang T, Higgins D. Dendritic growth induced by BMP-7 requires Smad1 and proteasome activity. *Journal of Neurobiology*. 2001;**48**(2):120-130
- [52] Garred MM, Wang MM, Guo X, Harrington CA, Lein PJ. Transcriptional responses of cultured rat sympathetic neurons during BMP-7-induced dendritic growth. *PLoS One*. 2011;**6**(7):e21754. DOI: 10.1371/journal.pone.0021754
- [53] Ghazalpour A, Bennett B, Petyuk VA, Orozco L, Hagopian R, Mungrue IN, Farber CR, Sinsheimer J, Kang HM, Furlotte N, Park CC, Wen PZ, Brewer H, Weitz K, Camp DG 2nd, Pan C, Yordanova R, Neuhaus I, Tilford C, Siemers N, Gargalovic P, Eskin E, Kirchgessner T, Smith DJ, Smith RD, Lusk AJ. Comparative analysis of proteome and transcriptome variation in mouse. *PLoS Genetics*. 2011;**7**(6):e1001393. DOI: 10.1371/journal.pgen.1001393
- [54] Nie L, Wu G, Culley DE, Scholten JC, Zhang W. Integrative analysis of transcriptomic and proteomic data: Challenges, solutions and applications. *Critical Reviews in Biotechnology*. 2007;**27**(2):63-75. DOI: 10.1080/07388550701334212
- [55] Dechant G, Barde YA. The neurotrophin receptor p75(NTR): Novel functions and implications for diseases of the nervous system. *Nature Neuroscience*. 2002;**5**(11):1131-1136. DOI: 10.1038/nn1102-1131
- [56] Horton A, Laramée G, Wyatt S, Shih A, Winslow J, Davies AM. NGF binding to p75 enhances the sensitivity of sensory and sympathetic neurons to NGF at different stages of development. *Molecular and Cellular Neurosciences*. 1997;**10**(3-4):162-172. DOI: 10.1006/mcne.1997.0650

- [57] Kenchappa RS, Zampieri N, Chao MV, Barker PA, Teng HK, Hempstead BL, Carter BD. Ligand-dependent cleavage of the P75 neurotrophin receptor is necessary for NRIF nuclear translocation and apoptosis in sympathetic neurons. *Neuron*. 2006;**50**(2):219-232. DOI: 10.1016/j.neuron.2006.03.011
- [58] Roux PP, Barker PA. Neurotrophin signaling through the p75 neurotrophin receptor. *Progress in Neurobiology*. 2002;**67**(3):203-233
- [59] Salama-Cohen P, Arevalo MA, Meier J, Grantyn R, Rodriguez-Tebar A. NGF controls dendrite development in hippocampal neurons by binding to p75NTR and modulating the cellular targets of Notch. *Molecular Biology of the Cell*. 2005;**16**(1):339-347. DOI: 10.1091/mbc.E04-05-0438
- [60] Shu YH, Lu XM, Wei JX, Xiao L, Wang YT. Update on the role of p75^{NTR} in neurological disorders: A novel therapeutic target. *Biomedicine & Pharmacotherapy*. 2015;**76**:17-23. DOI: 10.1016/j.biopha.2015.10.010
- [61] Underwood CK, Coulson EJ. The p75 neurotrophin receptor. *The International Journal of Biochemistry & Cell Biology*. 2008;**40**(9):1664-1668. DOI: 10.1016/j.biocel.2007.06.010
- [62] Bengoechea TG, Chen Z, O'Leary DA, Masliah E, Lee KF. P75 reduces beta-amyloid-induced sympathetic innervation deficits in an Alzheimer's disease mouse model. *Proceedings of the National Academy of Sciences of the United States of America*. 2009;**106**(19):7870-7875. DOI: 10.1073/pnas.0901533106
- [63] Wang KC, Kim JA, Sivasankaran R, Segal R, He Z. P75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. *Nature*. 2002;**420**(6911):74-78. DOI: 10.1038/nature01176
- [64] Courter LA, Shaffo FC, Ghogha A, Parrish DJ, Lorentz CU, Habecker BA, Lein PJ. BMP7-induced dendritic growth in sympathetic neurons requires p75(NTR) signaling. *Developmental Neurobiology*. 2016;**76**(9):1003-1013. DOI: 10.1002/dneu.22371
- [65] Roux PP, Bhakar AL, Kennedy TE, Barker PA. The p75 neurotrophin receptor activates Akt (protein kinase B) through a phosphatidylinositol 3-kinase-dependent pathway. *The Journal of Biological Chemistry*. 2001;**276**(25):23097-23104. DOI: 10.1074/jbc.M011520200
- [66] Sharma N, Deppmann CD, Harrington AW, St Hillaire C, Chen ZY, Lee FS, Ginty DD. Long-distance control of synapse assembly by target-derived NGF. *Neuron*. 2010;**67**(3):422-434. DOI: 10.1016/j.neuron.2010.07.018
- [67] Nykjaer A, Lee R, Teng KK, Jansen P, Madsen P, Nielsen MS, Jacobsen C, Kliemann M, Schwarz E, Willnow TE, Hempstead BL, Petersen CM. Sortilin is essential for proNGF-induced neuronal cell death. *Nature*. 2004;**427**(6977):843-848. DOI: 10.1038/nature02319
- [68] Redmond L, Ghosh A. The role of Notch and Rho GTPase signaling in the control of dendritic development. *Current Opinion in Neurobiology*. 2001;**11**(1):111-117
- [69] Van Aelst L, Cline HT. Rho GTPases and activity-dependent dendrite development. *Current Opinion in Neurobiology*. 2004;**14**(3):297-304
- [70] Yamashita T, Tucker KL, Barde YA. Neurotrophin binding to the p75 receptor modulates rho activity and axonal outgrowth. *Neuron*. 1999;**24**(3):585-593

- [71] Hamilton AM, Zito K. Breaking it down: The ubiquitin proteasome system in neuronal morphogenesis. *Neural Plasticity*. 2013;**2013**:196848. DOI: 10.1155/2013/196848
- [72] Oddo S. The ubiquitin-proteasome system in Alzheimer's disease. *Journal of Cellular and Molecular Medicine*. 2008;**12**(2):363-373. DOI: 10.1111/j.1582-4934.2008.00276.x
- [73] Sun L, Trausch-Azar JS, Ciechanover A, Schwartz AL. Ubiquitin-proteasome-mediated degradation, intracellular localization, and protein synthesis of MyoD and Id1 during muscle differentiation. *The Journal of Biological Chemistry*. 2005;**280**(28):26448-26456. DOI: 10.1074/jbc.M500373200
- [74] Kawabe H, Neeb A, Dimova K, Young SM Jr, Takeda M, Katsurabayashi S, Mitkovski M, Malakhova OA, Zhang DE, Umikawa M, Kariya K, Goebbels S, Nave KA, Rosenmund C, Jahn O, Rhee J, Brose N. Regulation of Rap2A by the ubiquitin ligase Nedd4-1 controls neurite development. *Neuron*. 2010;**65**(3):358-372. DOI: 10.1016/j.neuron.2010.01.007
- [75] Kim AH, Puram SV, Bilimoria PM, Ikeuchi Y, Keough S, Wong M, Rowitch D, Bonni A. A centrosomal Cdc20-APC pathway controls dendrite morphogenesis in postmitotic neurons. *Cell*. 2009;**136**(2):322-336. DOI: 10.1016/j.cell.2008.11.050
- [76] Litterman N, Ikeuchi Y, Gallardo G, O'Connell BC, Sowa ME, Gygi SP, Harper JW, Bonni A. An OBSL1-Cul7Fbxw8 ubiquitin ligase signaling mechanism regulates Golgi morphology and dendrite patterning. *PLoS Biology*. 2011;**9**(5):e1001060. DOI: 10.1371/journal.pbio.1001060
- [77] Izzi L, Attisano L. Regulation of the TGFbeta signalling pathway by ubiquitin-mediated degradation. *Oncogene*. 2004;**23**(11):2071-2078. DOI: 10.1038/sj.onc.1207412
- [78] Zhu H, Kavsak P, Abdollah S, Wrana JL, Thomsen GH. A smad ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation. *Nature*. 1999;**400**(6745):687-693. DOI: 10.1038/23293
- [79] Brennan AM, Suh SW, Won SJ, Narasimhan P, Kauppinen TM, Lee H, Edling Y, Chan PH, Swanson RA. NADPH oxidase is the primary source of superoxide induced by NMDA receptor activation. *Nature Neuroscience*. 2009;**12**(7):857-863. DOI: 10.1038/nn.2334
- [80] Kennedy KA, Sandiford SD, Skerjanc IS, Li SS. Reactive oxygen species and the neuronal fate. *Cellular and Molecular Life Sciences*. 2012;**69**(2):215-221. DOI: 10.1007/s00018-011-0807-2
- [81] Kowaltowski AJ, de Souza-Pinto NC, Castilho RF, Vercesi AE. Mitochondria and reactive oxygen species. *Free Radical Biology & Medicine*. 2009;**47**(4):333-343. DOI: 10.1016/j.freeradbiomed.2009.05.004
- [82] Colavitti R, Pani G, Bedogni B, Anzevino R, Borrello S, Waltenberger J, Galeotti T. Reactive oxygen species as downstream mediators of angiogenic signaling by vascular endothelial growth factor receptor-2/KDR. *The Journal of Biological Chemistry*. 2002;**277**(5):3101-3108. DOI: 10.1074/jbc.M107711200

- [83] Munnamalai V, Suter DM. Reactive oxygen species regulate F-actin dynamics in neuronal growth cones and neurite outgrowth. *Journal of Neurochemistry*. 2009;**108**(3):644-661. DOI: 10.1111/j.1471-4159.2008.05787.x
- [84] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology*. 2007;**39**(1):44-84. DOI: 10.1016/j.biocel.2006.07.001
- [85] Massaad CA, Klann E. Reactive oxygen species in the regulation of synaptic plasticity and memory. *Antioxidants & Redox Signaling*. 2011;**14**(10):2013-2054. DOI: 10.1089/ars.2010.3208
- [86] Cao X, Demel SL, Quinn MT, Galligan JJ, Kreulen D. Localization of NADPH oxidase in sympathetic and sensory ganglion neurons and perivascular nerve fibers. *Autonomic Neuroscience*. 2009;**151**(2):90-97. DOI: 10.1016/j.autneu.2009.07.010
- [87] Chandrasekaran V, Lea C, Sosa JC, Higgins D, Lein PJ. Reactive oxygen species are involved in bmp-induced dendritic growth in cultured rat sympathetic neurons. *Molecular and Cellular Neurosciences*. 2015;**67**:116-125. DOI: 10.1016/j.mcn.2015.06.007
- [88] O'Shea JJ, Gadina M, Kanno Y. Cytokine signaling: Birth of a pathway. *Journal of Immunology*. 2011;**187**(11):5475-5478. DOI: 10.4049/jimmunol.1102913
- [89] Iwamoto T, Oshima K, Seng T, Feng X, Oo ML, Hamaguchi M, Matsuda S. STAT and SMAD signaling in cancer. *Histology and Histopathology*. 2002;**17**(3):887-895. DOI: 10.14670/HH-17.887
- [90] Tang LY, Heller M, Meng Z, Yu LR, Tang Y, Zhou M, Zhang YE. Transforming growth factor-beta (TGF-beta) directly activates the JAK1-STAT3 axis to induce hepatic fibrosis in coordination with the SMAD pathway. *The Journal of Biological Chemistry*. 2017;**292**(10):4302-4312. DOI: 10.1074/jbc.M116.773085
- [91] Ulloa L, Doody J, Massague J. Inhibition of transforming growth factor-beta/SMAD signalling by the interferon-gamma/STAT pathway. *Nature*. 1999;**397**(6721):710-713. DOI: 10.1038/17826
- [92] Guo X, Chandrasekaran V, Lein P, Kaplan PL, Higgins D. Leukemia inhibitory factor and ciliary neurotrophic factor cause dendritic retraction in cultured rat sympathetic neurons. *The Journal of Neuroscience*. 1999;**19**(6):2113-2121
- [93] Guo X, Metzler-Northrup J, Lein P, Rueger D, Higgins D. Leukemia inhibitory factor and ciliary neurotrophic factor regulate dendritic growth in cultures of rat sympathetic neurons. *Brain Research. Developmental Brain Research*. 1997;**104**(1-2):101-110
- [94] Kim IJ, Beck HN, Lein PJ, Higgins D. Interferon gamma induces retrograde dendritic retraction and inhibits synapse formation. *The Journal of Neuroscience*. 2002;**22**(11):4530-4539
- [95] Kim EK, Choi EJ. Pathological roles of MAPK signaling pathways in human diseases. *Biochimica et Biophysica Acta*. 2010;**1802**(4):396-405. DOI: 10.1016/j.bbadis.2009.12.009

- [96] Plotnikov A, Zehorai E, Procaccia S, Seger R. The MAPK cascades: Signaling components, nuclear roles and mechanisms of nuclear translocation. *Biochimica et Biophysica Acta*. 2011;**1813**(9):1619-1633. DOI: 10.1016/j.bbamcr.2010.12.012
- [97] Guo X, Wang XF. Signaling cross-talk between TGF-beta/BMP and other pathways. *Cell Research*. 2009;**19**(1):71-88. DOI: 10.1038/cr.2008.302
- [98] Massague J. Integration of Smad and MAPK pathways: A link and a linker revisited. *Genes & Development*. 2003;**17**(24):2993-2997
- [99] Pera EM, Ikeda A, Eivers E, De Robertis EM. Integration of IGF, FGF, and anti-BMP signals via Smad1 phosphorylation in neural induction. *Genes & Development*. 2003;**17**(24):3023-3028. DOI: 10.1101/gad.1153603
- [100] Kim IJ, Drahushuk K, Kim W, Lein PJ, Andres DA, Higgins D. Extracellular signal-regulated kinases regulate dendritic growth in rat sympathetic neurons. *The Journal of Neuroscience*. 2004;**20**(8):2013-2037
- [101] Stachowiak MK, Fang X, Myers JM, Dunham SM, Berezney R, Maher PA, Stachowiak EK. Integrative nuclear FGFR1 signaling (INFS) as a part of a universal "feed-forward-and-gate" signaling module that controls cell growth and differentiation. *Journal of Cellular Biochemistry*. 2003;**90**(4):662-691. DOI: 10.1002/jcb.10606
- [102] Klimaschewski L, Meisinger C, Grothe C. Localization and regulation of basic fibroblast growth factor (FGF-2) and FGF receptor-1 in rat superior cervical ganglion after axotomy. *Journal of Neurobiology*. 1999;**38**(4):499-506
- [103] Horbinski C, Stachowiak EK, Chandrasekaran V, Miuzukoshi E, Higgins D, Stachowiak MK. Bone morphogenetic protein-7 stimulates initial dendritic growth in sympathetic neurons through an intracellular fibroblast growth factor signaling pathway. *Journal of Neurochemistry*. 2002;**80**(1):54-63
- [104] Andres DA, Shi GX, Bruun D, Barnhart C, Lein PJ. Rit signaling contributes to interferon-gamma-induced dendritic retraction via p38 mitogen-activated protein kinase activation. *Journal of Neurochemistry*. 2008;**107**(5):1436-1447. DOI: 10.1111/j.1471-4159.2008.05708.x
- [105] Altmann CR, Brivanlou AH. Neural patterning in the vertebrate embryo. *International Review of Cytology*. 2001;**203**:447-482
- [106] Chandrasekaran V, Zhai Y, Wagner M, Kaplan PL, Napoli JL, Higgins D. Retinoic acid regulates the morphological development of sympathetic neurons. *Journal of Neurobiology*. 2000;**42**(4):383-393
- [107] Melton KR, Iulianella A, Trainor PA. Gene expression and regulation of hindbrain and spinal cord development. *Frontiers in Bioscience*. 2004;**9**:117-138
- [108] Wilson L, Maden M. The mechanisms of dorsoventral patterning in the vertebrate neural tube. *Developmental Biology*. 2005;**282**(1):1-13. DOI: 10.1016/j.ydbio.2005.02.027

- [109] Ghzili H, Grumolato L, Thouennon E, Tanguy Y, Turquier V, Vaudry H, Anouar Y. Role of PACAP in the physiology and pathology of the sympathoadrenal system. *Frontiers in Neuroendocrinology*. 2008;**29**(1):128-141. DOI: 10.1016/j.yfrne.2007.10.001
- [110] Drahushuk K, Connell TD, Higgins D. Pituitary adenylate cyclase-activating polypeptide and vasoactive intestinal peptide inhibit dendritic growth in cultured sympathetic neurons. *The Journal of Neuroscience*. 2002;**22**(15):6560-6569
- [111] Quach DH, Oliveira-Fernandes M, Gruner KA, Tourtellotte WG. A sympathetic neuron autonomous role for Egr3-mediated gene regulation in dendrite morphogenesis and target tissue innervation. *The Journal of Neuroscience*. 2013;**33**(10):4570-4583. DOI: 10.1523/JNEUROSCI.5481-12.2013
- [112] Eldredge LC, Gao XM, Quach DH, Li L, Han X, Lomasney J, Tourtellotte WG. Abnormal sympathetic nervous system development and physiological dysautonomia in Egr3-deficient mice. *Development*. 2008;**135**(17):2949-2957. DOI: 10.1242/dev.023960
- [113] Vaillant AR, Zanassi P, Walsh GS, Aumont A, Alonso A, Miller FD. Signaling mechanisms underlying reversible, activity-dependent dendrite formation. *Neuron*. 2002;**34**(6):985-998
- [114] Naska S, Park KJ, Hannigan GE, Dedhar S, Miller FD, Kaplan DR. An essential role for the integrin-linked kinase-glycogen synthase kinase-3 beta pathway during dendrite initiation and growth. *The Journal of Neuroscience*. 2006;**26**(51):13344-13356. DOI: 10.1523/JNEUROSCI.4462-06.2006
- [115] Dedhar S, Williams B, Hannigan G. Integrin-linked kinase (ILK): A regulator of integrin and growth-factor signalling. *Trends in Cell Biology*. 1999;**9**(8):319-323
- [116] Arevalo JC, Chao MV. Axonal growth: Where neurotrophins meet Wnts. *Current Opinion in Cell Biology*. 2005;**17**(2):112-115. DOI: 10.1016/j.ceb.2005.01.004

