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Transforming Growth Factor-β in Normal Nociceptive Processing and Pathological Pain Models

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Summary The transforming growth factor-β (TGF-β) superfamily is a multifunctional, contextually acting family of cytokines that participate in the regulation of development, disease and tissue repair in the nervous system. The TGF-β family is composed of several members, including TGF-βs, bone morphogenetic proteins (BMPs) and activins. In this review, we discuss recent findings that suggest TGF-β function as important pleiotropic modulators of nociceptive processing both physiologically and under pathological painful conditions. The strategy of increasing TGF-β signaling by deleting "BMP and activin membrane-bound inhibitor" (BAMBI), a TGF-β pseudoreceptor, has demonstrated the inhibitory role of TGF-β signaling pathways in normal nociception and in inflammatory and neuropathic pain models. In particular, strong evidence suggests that TGF-β1 is a relevant mediator of nociception and has protective effects against the development of chronic neuropathic pain by inhibiting the neuroimmune responses of neurons and glia and promoting the expression of endogenous opioids within the spinal cord. In the peripheral nervous system, activins and BMPs function as target-derived differentiation factors that determine and maintain the phenotypic identity and circuit assembly of peptidergic nociceptors. In this context, activin is involved in the complex events of neuroinflammation that modulate the expression of pain during wound healing. These findings have provided new insights into the physiopathology of nociception. Moreover, specific members of the TGF-β family and their signaling effectors and modulator molecules may be promising molecular targets for novel therapeutic agents for pain management.

Keywords TGF-β · Activin · BMP · BAMBI · Nociception · Neuropathic pain · Pain

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Introduction

Pain is an unpleasant but indispensable sensation that warns the body to protect itself from severe damage. However, prolonged suffering from pain becomes a serious burden, and it is a major reason for which patients seek medical care and pharmacological treatment. Chronic pain is remarkably prevalent; it affects millions of people worldwide. Epidemiological surveys have revealed the staggering extent of chronic pain, its human costs and socio-economic impact, and also the paucity of effective methods for pain control [1, 2]. Poor pain control is the result of a deficit in our understanding of the mechanisms of chronic pain, which has limited our arsenal of pathogenesis-based analgesic therapies. However, there is considerable hope for the development of

new classes of analgesic drugs that target novel processes that contribute to clinically relevant pain. In this review, we highlighted recent results that identified TGF-βs as important pleiotropic modulators of nociceptive processing in physiological and pathological pain. The evidence indicates that specific members of the TGF-β family and their signaling effectors and modulators may be promising molecular targets for novel therapeutic agents for pain management.

The transforming Growth Factor-β Superfamily of Cytokines

The transforming growth factor-β (TGF-β) superfamily is a multifunctional, contextually acting family of cytokines that is comprised of more than 30 proteins. In mammals, these cytokines are grouped into different subfamilies: TGF-βs, bone morphogenetic proteins (BMPs), activins, growth and differentiation factors, anti-müllerian hormone and nodal. Members of the TGF-β family are produced as large precursor proteins within the cell, and they elicit biological responses as extracellularly secreted homo- or heterodimers. TGF-βs signal through serine (ser)-threonine (thr) kinase receptors of type I or activin receptor-like kinases (ALKs) and type II receptors (TβR-II). Ligand binding induces the formation of a stable receptor heterotetrameric complex that is composed of two receptors of each type. TGF-β family receptors are shared among ligands; only five type II receptors and seven type I receptors were described. Each member of the TGF-β superfamily binds to a characteristic combination of type I and type II receptors (Table 1). Type II receptors have intrinsic ser/thr protein kinase activity and transphosphorylate type I receptors at their GS domains (glycine- and serine-rich sequences), which stimulate their protein kinase activity; this phosphorylation is necessary and sufficient for TGF-β signaling. A type III receptor (TβR-III), also known as betaglycan, has also been described. However, type III receptors lack signaling domain and appear to act as co-receptors that enhance the binding of TGF-βs and BMPs to type I or type II receptors [3].

Canonical TGF-β Signaling

The activation of type I receptor kinases leads to the downstream propagation of the signal through the phosphorylation of intracellular receptor-activated Smad (R-Smad) proteins (Table 1, Fig. 1). Phosphorylated R-Smads interact with co-Smad4, which is a common co-factor of all TGF-β activin and BMP signaling pathways, to form Smad complexes that translocate to the nucleus and regulate gene transcription. The interaction of the type I receptor with specific R-Smad proteins is dependent on the ligand. TGF-βs and activins signal via Smad2 and Smad3, and BMPs signal through Smad1, Smad5 and Smad8. Smad complexes bind specific DNA sequences in the regulatory regions of numerous target genes, but the recruitment of Smads to a particular promoter and the specific transcriptional response that is elicited depend on the interaction of Smads with various DNA binding co-factors, co-activators and co-repressors [4]. This cooperative interaction between Smads and their DNA-binding partners confers a large spectrum of sequence specificities to TGF-β signaling and the potential for the integration or interaction of multiple signaling pathways within the cell [5].

Non-canonical TGF-β Signaling

TGF-β also alters cell behavior through the activation of Smad-independent pathways (Fig. 1). The mechanism that couples TGF-β to non-canonical effector systems and the biological consequences of this coupling remain poorly characterized. Several non-Smad signaling pathways with links to the TGF-β receptor complex are partially understood, including MAP kinases [TGF-β-activated kinase 1 (TAK1), Erk, p38 MAPK and c-Jun N-terminal kinase (JNK)], calcium-dependent phosphatase calcineurin-NFATc, growth and survival kinases PI3K/AKT/mTOR and small GTP-binding proteins Ras, RhoA, RhoB, Rac1 and Cdc42. The nuclear signals that are transmitted by non-Smad proteins can regulate transcription independently or synergize with the Smad proteins. Non-Smad proteins modulate the activity and signaling of Smad proteins. Furthermore, there is extensive crosstalk between Smads and

Table 1 Ligand–receptor–Smad relationships in the TGF-β family

Ligand	Type II receptors	Type I receptors	R-Smad	Co-Smad	I-Smads
TGF-βs	TβR-II	ALK5/TβR-I ALK1	Smad2 Smad3 Smad1 Smad5 Smad8	Smad4	Smad7
Activins	ActRIIA ActRIIB	ALK4/ActR1B ALK7/ActR1C	Smad2 Smad3	Smad4	Smad7
BMPs	BMPR-II ActRIIA ActRIIB	ALK3/BMPR-IA ALK6/BMPR-IB ALK1ALK2/ActR1A	Smad1 Smad5 Smad8	Smad4	Smad6 Smad7

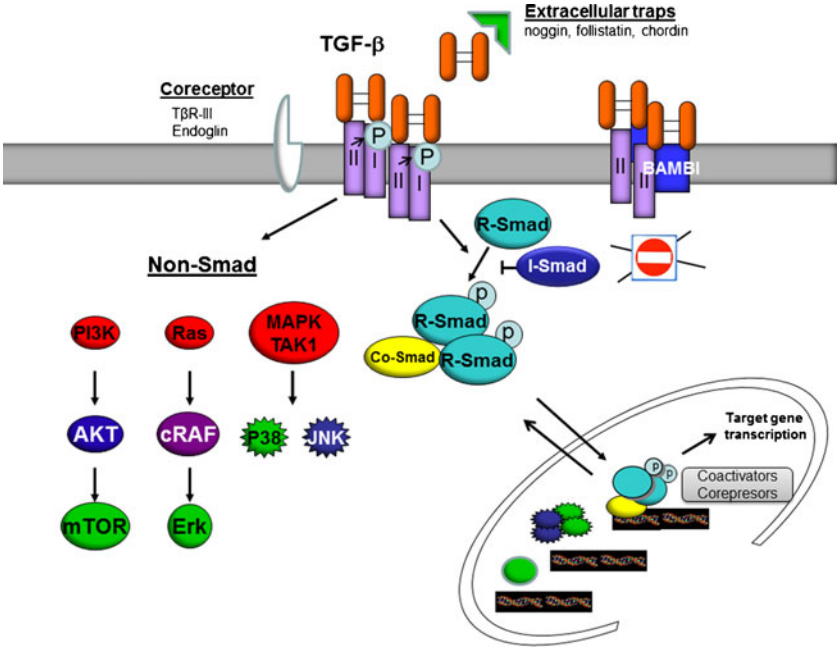

Fig. 1 The general mechanism of TGF-β receptor activation in Smad and non-Smad signaling pathways. The binding of ligands triggers heteromeric complex formation between TGF-β type I and type II receptors. Type I receptor is transphosphorylated and activated by the type II receptor kinase. The activated type I receptors phosphorylate R-Smads. Activated R-Smads form a complex with a common Smad4. R-Smad/Smad4 complexes translocate into the nucleus, where they regulate the transcription of target genes. The activation of R-Smads is inhibited by Smad6 or Smad7. TGF-βs also activate non-Smad signaling pathways, such as MAPK/p38/JNK and Ras/Erk-MAPK. TGF-β pathways are regulated by molecules that bind ligands in the extracellular space. Several membrane-associated proteins modulate the reception of TGF-β signals by the cell. BAMBI is a decoy receptor that prevents downstream signal transduction

kinase effectors with activity that is modulated by TGF-β ligands [6].

Regulation of TGF-β Signaling

The cell type- and context-dependent biological responses elicited by TGF-βs are determined by a variety of factors, including the extracellular concentration of the ligand, the presence and quantity of the complementary receptor on the target cell surface and the downstream signals that are activated [7]. The pleiotropic nature of TGF-βs is achieved through a tight control and a fine-tune of the strength, positioning and timing of signaling. Multiple mechanisms at every level, from the extracellular space to the transcriptional activity in the nucleus, cause remarkable context-specific gains or losses in TGF-β signaling [8, 9].

TGF-β pathways are often regulated by molecules that bind specific ligands in the extracellular space to limit their availability, control their diffusion from the producing cells, affect their transit through tissues or block their binding to receptors (Fig. 1). These regulatory molecules include a large set of specific extracellular diffusible proteins, such as noggin, follistatin, chordin, Dan, cerberus/caronte and gremlin [9].

Several membrane-associated proteins also modulate the cellular reception of TGF-β signals. The BMP and activin membrane-bound inhibitor (BAMBI) is structurally similar to type I receptors, but it lacks an intracellular kinase domain. Consequently, BAMBI acts as a decoy type I receptor that negatively modulates TGF-β/BMP/activin signaling by stably associating with type II receptors, which prevents the formation of active receptor complexes (Fig. 1) [10].

TGF-β-bound receptor complexes internalize by endocytosis. Clathrin-mediated internalization is required for Smad activation by the receptor complex, whereas caveolin- and lipid-raft-mediated endocytosis has been associated with receptor degradation. Protein associations that regulate the selection of the routing by the receptors would define the strength and duration of the signals and responses, and the receptor turnover [7].

The inhibitory Smad6 and Smad7 (I-Smads) are structurally divergent Smads which negatively regulate signaling strength and duration (Fig. 1). I-Smads bind to type I receptors and competitively inhibit R-Smad phosphorylation and the recruitment of phosphatases and Smurf ubiquitin ligases to downregulate receptor levels and activity [8].

A broad array of cytoplasmic and nuclear Smad interaction partners regulates Smad responses by (1) modulating their recruitment to the receptor complex, (2) controlling their phosphorylation, dephosphorylation and

sumoylation, (3) sequestering Smads from active signaling participation or (4) modulating the association of the R-Smad/Smad4 complex with transcription factors, co-activators and co-repressors and, subsequently, the Smad-dependent transcription [11], among other mechanisms.

Regulatory Roles of the TGF-β Family in Normal Nociceptive Processing and Pathological Pain Models

Nociception Overview

Nociception is a specialized form of sensory signaling that conveys information about potentially damaging stimuli to the central nervous system (CNS). The transduction of noxious stimuli originates at the peripheral axon terminals of high-threshold unmyelinated C or thinly myelinated Aδ primary sensory neurons that innervate the target tissue. Functional and molecular nociceptor heterogeneity is associated with the specific detection of distinct pain modalities. Nociceptor cell bodies reside in the dorsal root ganglia (DRG) of spinal nerves and the sensory ganglia of cranial nerves. The primary afferent projections from nociceptors transmit the signal from the periphery to the spinal cord dorsal horn, where they form synapses with second-order neurons in laminae I and V. The ascending fibers of second-order neurons project to third-order neurons in the thalamus and brainstem, which transmit the nociceptive information to higher brain structures that interpret the sensory-discriminative, affective-emotional or aversive dimensions of pain [12].

The release of inflammatory mediators within the wounded area amplifies peripheral nociceptor transduction. This "peripheral sensitization" is a form of stimulus-evoked functional plasticity, which normally occurs during the healing process, in which tissue damage enhances the excitability of nociceptors to protect the injured area by increasing pain sensitivity [13] .This physiological "nociceptive" pain normally disappears upon healing. However, pain persists in a state of chronic neuropathic pain after nervous system injuries in a small but significant percentage of the population [14].

In chronic neuropathic pain, a shift in the balance between the excitatory and inhibitory mechanisms that modulate spinal cord excitability heightens the response of dorsal horn neurons to incoming afferent signals and increases the output to the brain. This heightened sensitivity of spinal neurons is called "central sensitization" [15]. Sensitization of spinal cord nociceptive neurons contributes to hypersensitive pain behaviors such as allodynia (pain produced by normally innocuous stimuli), hyperalgesia (heightened response to noxious stimuli) and spontaneous pain [16]. However, no direct link between the experimental observation of spinal cord neuron hyperexcitability and the underlying mechanism of chronic pathological pain has been demonstrated [17]. Increasing evidence in the last decade has strengthened that chronic pain is a neuro-immune disorder that is caused by complex interactions between neurons, activated glial cells and inflammatory immune cells in the peripheral and CNS [18, 19]. This hypothesis suggests that the restoration of the balance between pro- and anti-inflammatory mechanisms may be used as a novel therapeutic approach to disrupt the development of chronic pain. Recent findings that support a relevant regulatory role for the TGF-β family of cytokines in acute physiological nociception, neuroprotection and anti-inflammation in models of chronic pathological pain are discussed in the following.

The Influence of BAMBI Deletion in Normal Nociception and in Models of Inflammatory and Neuropathic Pain

As mentioned previously, BAMBI is a kinase-deficient pseudoreceptor for TGF-βs which prevents downstream signal transduction [10]. BAMBI, together with several type I TGF-β receptors, is highly expressed in anti-nociception-relevant areas, such as the cingulate cortex, mesencephalic periaqueductal gray, spinal cord dorsal horn and DRG (Fig. 2) [20].

The induction of a gain in TGF-β signaling through the deletion of the BAMBI gene has been a valuable strategy for unraveling the involvement of the TGF-β family in pain control [20]. BAMBI-KO mice display attenuated nocifensive responses in acute pain models, regardless of the modality of the noxious stimuli (e.g. thermal, mechanical and chemical/inflammatory). Moreover, BAMBI-KO mice develop less mechanical allodynia in models of chronic neuropathic pain (Fig. 2). The hypoalgesic phenotype of BAMBI-KO mice can be reversed by the opioid antagonist naltrexone, which is indicative of an enhanced activity of the endogenous opioid system. Spinal cords from BAMBI-KO mice display higher expression levels of endogenous opioid peptide precursors, including β-endorphins (proopiomelanocortin: POMC) and enkephalins (proenkephalin: PENK), compared to wild-type mice. Opioid peptide precursors are under the transcriptional control of TGF-β family members in cultured cells [21, 22]. Furthermore, exogenous TGF-βs induce the expression of *POMC* and *PENK* in cultured spinal cord explants [20]. Therefore, an increase in TGF-β signaling activity may lead to an increase in the transcription, expression and synaptic release of endogenous opioids and a hypoalgesic phenotype.

The TGF-β Subfamily Protects Against Nerve-Injury-Induced Neuropathic Pain

Pharmacological approaches have contributed to knowing the specific contribution of individual TGF-β family members to

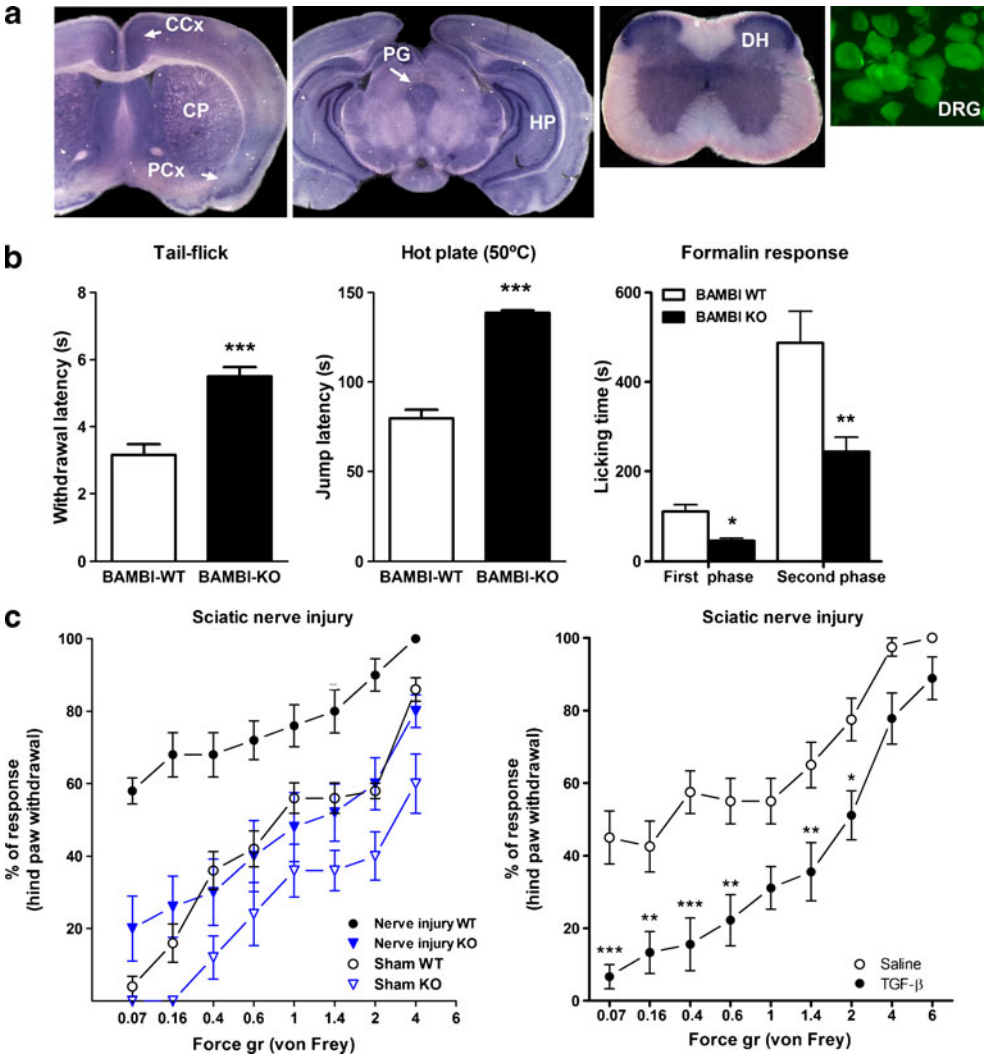

Fig. 2 BAMBI expression in the mouse CNS and the hypoalgesic phenotype of BAMBI-KO mice. **a** Localization of BAMBI mRNA in brain and spinal cord sections (in situ hybridization using digoxigenin-labeled riboprobes) and BAMBI protein in DRG neurons (immunofluorescence). *CCx* cingulate cortex, *PCx* pyriform cortex, *CP* caudate-putamen nucleus, *HP* hippocampus, *PG* mesencephalic periaqueductal gray, *DH* dorsal horn of the spinal cord, *DRG* dorsal root ganglion. **b** Responses of BAMBI-KO and WT mice to acute painful stimuli. Two models of thermal nociception were used: The tail-flick test that examines spinal-mediated responses, and the hotplate test that examines both spinal and supraspinal-mediated responses. Chemical/inflammatory pain was induced by 20-μl intraplantar injection of a 2% formalin solution in the left hind paw, and the cumulative time spent licking the paw was recorded within the first 5 min (first phase) and from 20 to 50 min after injection (second phase). Data are means±SEM. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ versus BAMBI-WT mice (two-tailed Student's *t* test). **c** Development of neuropathic pain in response to crush injury of sciatic nerve. The *left panel* displays the mechanical allodynia evaluated with von Frey monofilaments on day 14 after nerve injury. Values are mean±SEM percentage of hind paw withdrawals elicited by mechanical stimuli of increasing strength in wild-type and BAMBI-KO mice subjected to sham operation or sciatic nerve injury. BAMBI-KO mice, compared with their wild-type littermates, are less sensitive to mechanical stimuli under basal conditions and develop attenuated allodynia following sciatic nerve injury. The *right panel* shows the anti-allodynic effect induced by a 2-week subcutaneous infusion of recombinant TGF-β1 in mice subjected to sciatic nerve injury, compared with saline. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ versus saline (Bonferroni test)

the control of nociceptive transmission. Echeverry et al. [23] demonstrated the protective role of TGF-β1 against nerve-injury-induced neuropathic pain. Thus, sustained intrathecal infusion of recombinant TGF-β1 during partial ligation of the sciatic nerve in rats significantly attenuates the development of mechanical allodynia and thermal hyperalgesia for 14 days. More importantly, from a therapeutic point of view, TGF-β1 also produces a significant reduction in previously established hyperalgesia. Because TGF-β can cross the blood–brain barrier (BBB) [24], we analyzed its effectiveness after systemic administration. As shown in Fig. 2, 2-week subcutaneous infusion of recombinant TGF-β1 significantly attenuates the development of mechanical allodynia in mice.

Echeverry et al. [23] attributed the biological effects of TGF-β1 to a milder neuroinflammatory response to injury in the spinal cord. These authors provided evidence that TGF-β1 exerted neuroprotective effects, inhibited the activation of spinal microglia and astrocytes and decreased the upregulation of pro-inflammatory cytokines, such as IL-1β and IL-6, within the spinal cord. These results are consistent with the anti-inflammatory properties of this cytokine [25, 26].

The contribution of peripheral mechanisms to the effects of TGF-β is also important because several TGF-β family members (mainly activin and BMPs) exert pro-nociceptive effects on peripheral nociceptors. Our results indicate that 2 weeks of systemic administration of a TGF-β neutralizing antibody, which does not cross the BBB, significantly enhanced the development of mechanical allodynia in mice after sciatic nerve injury (our unpublished observations). These findings support the idea that TGF-β also exerts peripheral anti-nociceptive effects and helps prevent peripheral nociceptor hypersensitization following nerve injury.

The capability of TGF-β to maintain the integrity of the BBB has also been suggested as a protective mechanism against the development of pathological pain following peripheral inflammatory [27, 28] or neural injuries [29]. Systemic or intrathecal treatment with recombinant TGF-β1 prevents carrageenan- and nerve-injury-induced changes in endothelial tight junctions, as well as the functional disruption of the BBB. As a consequence, the extravasation of proteins, cytokines and a variety of inflammatory mediators and the infiltration of peripheral inflammatory cells in the spinal cord is significantly reduced. The mechanism involves ALK 5 receptors and the canonical Smad2/3 signaling pathway [27, 29].

In summary, the TGF-β subfamily protects against pathological pain development by pleiotropic mechanisms. TGF-β appears to promote the expression of endogenous opioids and inhibit the neuroimmune responses of glial cells and neurons in the spinal cord following peripheral injuries. In addition, undetermined peripheral mechanisms also contribute to its anti-allodynic effect. Even at this early stage of investigation, the evidence suggests that the modulation of TGF-β signaling could be used as a novel pharmacological strategy for the control and treatment of chronic pain.

Neuropathic Pain is Dissimilarly Modulated by Individual Members of the BMP Subfamily

The role of the BMP family in the processing of nociceptive inputs within the CNS has been scarcely addressed, and different results were obtained depending on the specific BMP analyzed. Recent studies indicate that the use of recombinant BMP2 as bone inductor during surgical spinal fusion elicits a profound Smad-mediated signaling response in the spinal cord and local DRG [30]. The direct access of BMP2 to the nervous system triggers a neuroinflammatory response that worsens the neurologic recovery and produces postoperative allodynia in rats [31]. This pro-algesic effect could have a clinical correlate as patients that received recombinant BMP2 were more likely to report postoperative radicular pain compared with patients whose fusion surgery did not include use of the protein [32] However, the presence of a dural tear that would favor BMP2 diffusion into the spinal cord parenchyma did not increase the risk of radiculitis development or impair the neurologic recovery in a series of patients [33].

BMP4 has been reported to exert an indirect protective effect against hyperalgesia development. Neuropathic pain is a common harmful side effect of neuroepithelial stem cell transplantation therapy for spinal cord injury [34, 35]. The pretreatment of glial precursor cells with BMP4 ($GDAs^{BMP}$) prevents the development of thermal hyperalgesia and mechanical allodynia in rats. Such protection may be related with the absence of dorsal horn sprouting of CGRP-immunoreactive C-fiber in mice treated with $GDAs^{BMP}$ [36]. In addition, the activation of BMP signaling pathways in $GDAs^{BMP}$ promotes the expression of glutamate transporter 1 and AKAP12 [37], which are genes relevant to preventing inflammatory and neuropathic pain [38] and to maintaining the BBB integrity [39]. By contrast, intrathecal delivery of BMP4 cDNA incorporated into an adenovirus vector does not modify somatosensory perceptions in mice subjected to spinal cord injury, while it promotes sensory axon regeneration [40]. Similarly, BMP7 overexpression in the sciatic nerve, DRG and spinal cord neurons by adenoviral gene transfer improves the functional recovery after injury but does not affect the development of neuropathic pain in rats [41].

Activin and BMP Retrograde Signals Modulate the Expression of the Neuropeptide CGRP by Nociceptors and Contribute to Neuroinflammatory Pain

The terminal differentiation of many developing neurons occurs after the innervation of their target cells. This differentiation is triggered by extrinsic, target-derived retrograde signals that are transduced by presynaptic cognate receptors. Target tissues contain neurotrophic growth factors that support neuronal survival and differentiation factors that are critical for the maturation of axonal projections and the acquisition of neuronal traits, such as the expression of distinct combinations of neurotransmitters [42, 43]. Epidermal keratinocytes have a dynamic neurochemical organization that plays a direct role in the modulation of sensory ending functions [44]. Retrograde

communication between the skin and nociceptive neurons by members of the TGF-β family regulates the phenotypic identity of nociceptors and the circuit assembly. This communication also contributes to the plasticity that effects dynamic changes in nociceptor sensitivity in physiological and pathological situations.

TGF-β receptors are broadly expressed by embryonic and adult neurons in trigeminal dorsal root sensory ganglia, which indicates the potential TGF-β sensitivity of these cells [45–48]. In addition, members of the TGF-β family are present in major nociceptor targets such as the skin [46, 49, 50].

A well-documented consequence of target-derived TGF-β signals is the specification of the neurotransmitter phenotype of neuropeptide-containing DRG neurons. Glutamate is the major neurotransmitter released by nociceptors, but many nociceptive neurons also contain neuropeptide co-transmitters that help to transmit and modulate nociceptive inputs. Calcitonin gene-related peptide (CGRP) and substance P (SP) are the main neuropeptides that are expressed by an important subset of nociceptors that innervate skin and viscera [51]. Embryonic nociceptors contain no detectable CGRP mRNA or protein until their axons contact target tissues and the peripheral connections are functional, which indicates that peptidergic phenotype specification in nociceptive neurons is dependent on retrograde signals from targets [52, 53].

Studies in vitro have shown that skin-cell-derived factors induce de novo CGRP expression in embryonic DRG neurons that are isolated before peripheral target contact, and this effect is antagonized by anti-activin A antibody or the activin and BMP inhibitory protein, follistatin [45, 54–56]. Furthermore, the addition of recombinant activin A, BMP2, BMP4, BMP6 or BMP7 to cultured embryonic neurons that were dissociated from DRG induces a strong increase in the expression of CGRP by the specific subset of unmyelinated C-fiber peptidergic neurons. This effect involves the canonical Smad pathway [45, 56]. In postnatal DRG cultures, recombinant activin increases CGRP expression [56–59], while TGF-β1 and TGF-β2 increase the levels SP [59]. The effect of these cytokines on neuropeptide expression is synergistic with NGF, which led the authors to hypothesize that a cooperative interaction between Smads and NGF-dependent transcription factors further increases neuropeptide transcription [59]. Overall, these data support a role for TGF-β family members as target-derived differentiation factors that contribute to the specification of the functional identity of neuropeptide-containing neurons during development. Moreover, sensory neurons remain sensitive to these cytokines during adulthood [46, 54, 58, 59].

The contribution of TGF-β retrograde signals to the modulation of CGRP transcription by nociceptors in vivo is highly relevant. CGRP conveys pain information from nociceptors to second-order neurons in the spinal cord, and it is also an important mediator of normal inflammatory pain response to injuries [60, 61]. CGRP is also involved in the development of hyperalgesia in inflammation- or nerve-injury-induced chronic pain [62]. Studies in vivo suggest that the conditional overexpression of BMP4 in mouse skin keratinocytes reduces the number of non-peptidergic neurons in sensory ganglia and the density of target innervation, but the nerve endings from CGRP peptidergic neurons were markedly increased [63].This phenotype is consistent with the aforementioned effect of BMP signaling on CGRP expression in vitro [45]. The phenotypic changes that are induced by BMP4 overexpression are more prominent with increasing age, which suggests that the same retrograde communication between skin cells and sensory nerve endings is maintained in adults [62].

Activin is involved in the inflammatory response that is associated with the healing and repair of injured tissues in experimental adult animal models [56, 64–67]. Although the basal level of activin A in the skin is notably low, its expression is strongly increased following cutaneous excisional wound injury or under inflammation produced by complete Freund's adjuvant [56, 68, 69]. Under these experimental conditions, the CGRP-containing sensory neurons that innervate the skin wound region receive an important trophic influence from activin released by keratinocytes and inflammatory cells, which strongly enhances the expression of CGRP and other neuropeptides [56]. Furthermore, activin injection in the skin of adult naïve rats produces a tactile allodynia to mechanical stimulation that is associated with an increase in the proportion of CGRP-immunoreactive DRG neurons innervating the area of injection [57]. In addition, β-CGRP is released by skin keratinocytes, and its expression is regulated by autocrine/paracrine BMP signaling [62]. Importantly, β-CGRP is markedly increased in the skin of patients and mice with chronic inflammatory or neuropathic pain, which suggests an additional contribution of keratinocyte-dependent release of β-CGRP in regulating nociceptor plasticity under conditions of pathological pain [62].

The CGRP released by peripheral nerve endings and keratinocytes causes neurogenic vasodilatation, extravasation of serum factors important for wound healing, mast cell degranulation and platelet activation. The resulting accumulation of pro-inflammatory factors in the injured area plays an important role in the development and maintenance of peripheral sensitization of nociceptors and the phenotypic changes of DRG neurons that augment central transmission and peripheral sensitization. In addition, CGRP released by primary afferents contributes to the central sensitization of second-order neurons [70, 71].The sensitization of the nociceptive system contributes to pain

hypersensitivity at the site of tissue damage and inflammation. Therefore, the modulation of CGRP-containing nociceptors by retrograde activin signaling promotes the heightened nocifensive behaviors that protect against further damage during the normal tissue healing process. An understanding of the role of activin and other TGF-β family members in the CGRP-dependent sensitization of nociceptive signaling could provide further insights into the pathophysiology of highly prevalent pain diseases that involve this neuropeptide, such as migraine and other primary headaches [72].

Activin Sensitizes TRPV1 Ionic Currents in DRG Neurons and Produces Thermal Hyperalgesia in Mice

Transient receptor potential vanilloid 1 (TRPV1) is a non-selective cation channel that is expressed by polymodal nociceptors from somatic and visceral afferents, which are gated by noxious heat and irritant vanilloids, such as capsaicin and extracellular protons, and these nociceptors play a critical role in the development of heat hyperalgesia after inflammation [73] and visceral hyperalgesia in inflammatory bowel disease and rectal hypersensitivity [74]. Electrophysiological recordings of cultured DRG neurons suggest that recombinant activin A acutely potentiates capsaicin-induced ionic currents through TRPV1 by a mechanism that involves ALK4 receptors [48]. The epidermal injection of activin induces a rapid and short-lasting thermal hyperalgesia in wild-type mice, but this response is absent in TRPV1 null-mutant mice. Therefore, the positive modulation of TRPV1 by activin may be a novel mechanism underlying sustained inflammatory pain. Functionally, TRPV1 activation depends on a complicated balance of phosphorylation and dephosphorylation signals. Pro-nociceptive inflammatory mediators increase TRPV1 phosphorylation by the activation of multiple kinases, which sensitizes the TRPV1 response to noxious heat and regulates inflammatory hyperalgesia [75]. The non-canonical activation of PKCε probably mediates acute TRPV1 sensitization by activin. In cultured DRG neurons, activin induces the translocation of PKCε from the cytosol to the plasma membrane, and this effect is prevented by the blockade of ALK4 receptors. Moreover, a PKCε translocation inhibitor peptide prevents activin-A-induced TRPV1 sensitization. Other protein kinases, such as PKA, MAPK, PI3K, c-src and other PKC isoforms, are not likely to play a role in TRPV1 sensitization because selective inhibitors of these kinases are ineffective [48].

From a therapeutic point of view, pharmacological approaches for the modulation of TRPV1 receptor function offers a promising means of pain relief at the nociceptor level. TRPV1 agonists produce a long-lasting desensitization of peripheral nerve terminals to noxious stimuli (nociceptor "defunctionalization"), which is the mechanism that underlies the proven efficacy of TRPV1 agonists, such as capsaicin for neuropathic pain relief. TRPV1 antagonists also alleviate hyperalgesia associated with inflammatory pain [76]. Because activin has a sensitizing effect on TRPV1 channels, it would be useful to assess whether the inhibition of peripheral activin signaling interferes with the development of hyperalgesia under painful pathological conditions by reducing TRPV1 currents and whether exogenous activin induces nociceptor defunctionalization and long-lasting anti-nociception.

Conclusions and Perspectives

Members of the TGF-β family play crucial roles in the pathophysiology of nociception, both at the peripheral sensory neurons and in the CNS. In models of inflammatory and neuropathic pain, the TGF-β subfamily provides protection against the neuroinflammatory responses, prevents the disruption of the BBB integrity and favors the release of opioid analgesic mediators. The effects of the BMP family in the central processing of nociceptive inputs are largely unknown, and different results have been reported depending on the specific BMP analyzed. Recombinant BMP2 triggers a neuroinflammatory response that produces allodynia. Conversely, BMP4 could exert an indirect protective effect against hyperalgesia development. In the peripheral nervous system, activin and, presumably, BMPs promote heightened nocifensive behaviors that protect against further damage during the process of tissue healing. Activin also has a sensitizing effect on TRPV1 channels in DRG neurons, which elicits thermal hyperalgesia.

Further work is needed to identify the most promising components of the TGF-β signaling pathways which might serve as viable therapeutic targets for the treatment of pathological pain. Also, due to the pleiotropic effects of these cytokines, a detailed investigation of the off-target effects that can potentially lead to unwanted toxicities is necessary before successful development of TGF-β-based therapeutic strategies.

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