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New insights into the mechanisms of itch: are pain and itch controlled by distinct mechanisms?

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Abstract

Itch and pain are closely related but distinct sensations. They share largely overlapping mediators and receptors, and itch-responding neurons are also sensitive to pain stimuli. Itch-mediating primary sensory neurons are equipped with distinct receptors and ion channels for itch transduction, including Mas-related G protein-coupled receptors (Mrgprs), protease-activated receptors (PARs), histamine receptors, bile acid receptor (TGR5), toll-like receptors (TLRs), and transient receptor potential subfamily V1/A1 (TRPV1/A1). Recent progress has indicated the existence of an itch-specific neuronal circuitry. The MrgprA3-expressing primary sensory neurons exclusively innervate the epidermis of skin and their central axons connect with gastrin-releasing peptide receptor (GRPR)-expressing neurons in the superficial spinal cord. Notably, ablation of MrgprA3-expressing primary sensory neurons or GRPR-expressing spinal cord neurons results in selective reduction in itch but not pain. Chronic itch results from dysfunction of the immune and nervous system and can manifest as neural plasticity, despite the fact that chronic itch is often treated by dermatologists. While differences between acute pain and acute itch are striking, chronic itch and chronic pain share many similar mechanisms, including peripheral sensitization (increased responses of primary sensory neurons to itch and pain mediators), central sensitization (hyperactivity of spinal projection neurons and excitatory interneurons), loss of inhibitory control in the spinal cord, and neuro-immune and neuro-glial interactions. Notably, painful stimuli can elicit itch in some chronic conditions (e.g., atopic dermatitis) and some drugs for treating chronic pain are also effective in chronic itch. Thus, itch and pain have more similarities in pathological and chronic conditions.

Keywords

Central sensitization; neuro-immune interaction; nociceptor; peripheral sensitization; pruritus; pruriceptor

Introduction

Itch (pruritus) was first defined by a German physician Samuel Hafenreffer as “an unpleasant sensation that elicits the desire or reflex to scratch” [61]. Although this definition is still valid today, it also reflected an incomplete understanding of the underlying mechanisms of itch. Itch can be induced acutely, such as mosquito bite, and this itch only lasts minutes and is inhibited by scratching the affected area [34]. Itch was considered as a sub-modality of pain, because two sensations share many similarities (Box 1). Like acute

pain, acute itch is protective and serves as a warning signal for potential tissue damage caused by invader such as parasite [126]. However, chronic itch leads to sleep disruption and severe skin lesions [75]. Chronic itch commonly occurs in patients with skin diseases, and also is a comorbidity of other systemic disorders, such as liver and kidney diseases, HIV/AIDS, as well as metabolic disorders [151]. Notably, chronic itch is underappreciated and difficult to manage. Although antihistamines are the first choice for anti-itch treatment, most chronic itch conditions are resistant to antihistamines, possibly due to dominant contributions of the histamine-independent mechanisms. In chronic itch patients, scratching-evoked sensation can be perceived as itch and produce an “itch-scratch-itch” cycle to exacerbate itch. In some chronic itch conditions such as atopic dermatitis, even painful stimuli could be perceived as itch [57;60;86]. Continuous scratching not only increases the risk of skin infection, but also disrupts the social life of patients [75;119;151].

Box 1**Similarities and differences between itch and pain**

	Similarities	Differences
Acute states	<ul style="list-style-type: none"> • Both are unpleasant sensations. • Both are protective in the normal conditions. • Both are mediated by capsaicin-sensitive C-fibers. • Both are regulated by proinflammatory mediators (e.g., histamine, 5-HT, ET-1 & PGs). • Both employ similar ion channels, such as TRPV1 and TRPA1. • Both may use glutamate and neuropeptides (SP and CGRP) as neurotransmitters. 	<ul style="list-style-type: none"> • Itch stimuli evoke scratching while pain stimuli induce withdrawal reflex. • Itch origins from the skin or mucosal surfaces, while pain origins from almost all body parts. • Itch is inhibited by painful stimuli. • Inhibition of pain by analgesic such as morphine can provoke itch. • Only a small portion of primary nociceptive neurons respond to itch. • Itch is mediated by MrgA3+ primary sensory neurons and GRPR+ dorsal horn neurons.
Chronic states	<ul style="list-style-type: none"> • Both are debilitating conditions in patients and pets. • Pain stimuli can be perceived as itch sensation in atopic dermatitis. • Both are enhanced by peripheral sensitization, elicited by inflammatory mediators (e.g., NGF, TNF-α) and TRPA1/V1. • Both are potentiated by central sensitization, such as loss of inhibitory control and enhanced excitatory synaptic transmission. • Both are regulated by immune cells and glial cells in the PNS and CNS. • Both can be reduced by gabapentin, pregabalin, local 	<ul style="list-style-type: none"> • Itch is mediated by specific cytokines (IL-2, IL-13 or IL-31), while pain is elicited by various cytokines (e.g., IL-1, IL-6, etc) and chemokines (e.g., CCL2, CCL5, CXCL1). • μ-opioid receptor agonists induce itch while μ-opioid receptor antagonists reduce itch. • δ-opioid receptor agonists reduce itch while δ-opioid receptor antagonists induce itch.

	Similarities	Differences
	anesthetics, clonidine, and antidepressants.	

To date, our understanding for chronic itch associated with systemic diseases (e.g., renal, liver or metabolic diseases) is still incomplete, due to the lack of suitable animal models of chronic itch. Several animal models were developed for dermatitis-induced chronic itch, including dry skin model induced by a mixture of acetone/ether (1:1) and water (AEW) [3;4;106], toxic contact dermatitis model induced by diphenylcyclopropanone (DCP) [134;145], and allergic contact dermatitis model induced by 2,4-Dinitro-1-fluorobenzene (DNFB) [130]. In addition, an inbred mouse line (NC/Nga mice) was used to study atopic dermatitis-related itch [50;116;146]. Development of animal models to mimic clinical itch conditions is essential to elucidate the molecular mechanisms of chronic itch [80], which are different from that of acute itch (Box-2)

Box 2

Outstanding questions

- What are the neurotransmitters for itch transmission? What are the roles of GRP, SP, CRGP, and glutamate in spinal cord itch transmission?
- Are GRPR-expressing neurons projection neurons or interneurons?
- What are the neurochemical properties of the Bhlhb5-expressing inhibitory neurons in the spinal cord?
- What are the endogenous ligands for MrgprA3/C11/D and TLR3/7? How are Mrgprs and TLRs regulated under chronic itch conditions?
- Are there itch-specific fibers in human that are equivalent to MrgprA3+ itch fibers in mice?
- How do glial cells control itch? Do glial cells release different mediators to regulate pain and itch transmission in the spinal cord?
- Are there itch mediators that are only produced in chronic conditions and act when pruroceptors are up-regulated?
- Are there suitable animal models for clinical itch? Although acute itch models are useful for studying itch physiology, the chronic itch models that recapitulate clinical itch conditions are urgently needed for studying pathological itch.

In this review, we provide new insights into distinct molecular mechanisms of itch, especially nonhistaminergic itch signaling, mediated by Mas-related G protein-coupled receptor (Mrgprs), protease-activated receptors (PARs), toll-like receptors (TLRs), oxidative stress, transient receptor potential cation channel subfamily V1/A1 (TRPV1/A1), and interleukin-31 (IL-31). We also discuss recent evidence for the existence of an itch-specific neuronal pathway consisting of MrgprA3-expressing primary sensory neurons and GRPR-expressing spinal dorsal horn neurons. We further discuss the mechanisms underlying chronic itch [33], especially the crosstalk of the itch and pain pathways (labeled lines) in physiological and pathological conditions. While the differences of itch and pain are striking in acute conditions, itch and pain share many similar mechanisms in chronic conditions. Pain stimuli could potentiate itch in pathological conditions as a result of neural plasticity.

Characterization of itch

Itch is exclusively sensed as an irritation in the skin, while pain can be experienced from almost all the body parts [157]. Based on the distinct origin, itch is classified into 4 categories: (1) pruriceptive itch, defined as itch that originates from the skin. Itch is perhaps the most common symptom in dermatology, as a result of dry skin, atopic eczema, psoriasis, urticaria, scabies or other inflammatory skin diseases [105]; (2) neuropathic itch, caused by nerve damage, such as itch associated with post-herpes zoster neuropathy, brain tumors and multiple sclerosis [115]; (3) neurogenic itch, caused by dysfunction of the nervous system in the absence of peripheral neuropathy, such as itch related to cholestasis [21]; and (4) psychological or psychiatric itch, such as itch related to psychological abnormalities (e.g., obsessive-compulsive disorders [157]). For comparison, pain also can be divided into 4 categories: 1) nociceptive pain, caused by activation of peripheral nerve fibers by noxious stimulation; 2) inflammatory pain, pain associated with tissue damage and inflammation; 3) neuropathic pain, caused by damage or disorder affecting of the nervous system; 4) Psychogenic pain, caused by mental, emotional or behavioral factors [155]. Depending on the duration, itch and pain can also be characterized as acute and chronic itch and pain in humans. Acute itch lasts minutes to days, whereas chronic itch lasts more than 6 weeks. On the other hand, acute pain lasts less than 30 days, but chronic pain can last more than six months [61]. Notably, the duration of chronic itch and pain could be much shorter in experimental animals (e.g., rodents), in part due to short life expectancy of rodents.

Peripheral mechanisms of itch

Itch mediators and receptors

Itch and pain sensations are mediated by primary sensory neurons, whose cell bodies are located in dorsal root ganglia (DRG) or trigeminal ganglia (TG) [61]. Traditionally, noxious chemicals can be classified as algogens or pruritogens. However, increasing evidence shows that most noxious chemicals are not specific for itch or pain, except for the allyl isothiocyanate (AITC) that only elicits pain [126;139]. Many itch-inducing mediators (Figure 1), including histamine, serotonin (5-HT), and endothelin-1 (ET-1) also induce pain. On the other hand, many receptors or ion channels such as TRPV1 and TRPA1 that are involved in pain transduction also play pivotal roles in itch transduction [22].

Histamine—Histamine is one of the best known itch mediators. However, antihistamines are ineffective in many chronic itch conditions, such as itch associated with atopic dermatitis, kidney and liver diseases, suggesting the existence of histamine-independent mechanism in chronic conditions. Thus, itch is often characterized as histamine-dependent (histaminergic) and histamine-independent (nonhistaminergic) itch [63]. Histamine is naturally synthesized from the amino acid histidine and is released from mast cells in skin when these cells are activated by allergens or inflammatory mediators [136]. Free nerve fibers in the skin are responsible for detecting pruritogens [125]. A subgroup of mechano-insensitive unmyelinated C-fibers are responsible for histamine-induced itch in humans [132]. Histamine receptors are members of the G-protein-coupled receptors (GPCRs), and four subtypes of histamine receptors (H1–H4) have been identified to date [58;136]. Both H1R and H4R have been detected on DRG neurons and are involved in histamine-induced itch [52]. H1R is coupled with Gq/G₁₁ to induce activation of phospholipase C 3 (PLC 3), leading to increase of intracellular Ca²⁺ in DRG neurons via TRPV1 [20;38]. Histamine also activates the phospholipase A2 (PLA2), lipoxygenase, and TRPV1 in primary sensory neurons to induce itch [70;136;137;150] (Figure 1). H1R antagonists have been widely used to relieve itching and reduce size of flares. H4R may also play a role in itch. In mouse model of atopic dermatitis, histamine activates H4R to cause itching, and H4R antagonists decrease

itching and inflammation [30]. However, the H4R-mediated signaling in primary sensory neurons still needs to be determined.

5-HT—The role of 5-HT in itch may be species-dependent. In rats, 5-HT induces pure itch behavior, while in mouse and human it may evoke mixed pain and itch sensations [103]. Skin mast cells are likely a primary source of 5-HT. 5-HT induces itch mainly via 5-HT₂ receptors [156], which are coupled to Gq/11 to activate PLC, especially PLC β 3 [62], leading to the activation of the mitogen-activated protein kinase (MAPK) and PKC in sensory neurons [103].

ET-1—ET-1 is a 21 amino acids peptide from skin keratinocytes and endothelial cells. Intradermal application of ET-1 causes mixed pain and itch behaviors in mice [45]. ET-1 binds to two subtypes of GPCRs, ETA and ETB, and ETA was thought to regulate both itch and pain [104]. ETA couples to Gs to raise cAMP levels and also activates PLC. Interestingly, PLC β 3 and TRPV1 are not required for ET-1-induced itch in mice [62].

Bile acids—A recent study indicated that bile acids, which are elevated in the plasma and also accumulated in skin in patients with cholestasis [21], cause itch by activating the GPCR TGR5 (also called GPR131 or GPBAR1) [8]. TGR5 was detected in a subpopulation of DRG neurons that co-express gastrin-releasing peptide (GRP), TRPA1 and TRPV1. Intradermal injection of bile acids and other TGR5 agonist evoked TGR5-dependent scratching behavior in mice. Interestingly, this behavior also depends on GRP and opioid [8]. Bile acids cause hyperexcitability of DRG neurons via TGR5 activation. Thus, it is plausible that bile acids activate TGR5 on sensory nerves to stimulate the release of neuropeptides (e.g., GRP and opioid peptide) in the spinal cord for eliciting itch transmission. Although bile acids levels are elevated in circulation and skin in cholestatic patients, these levels do not necessarily correlate with itch intensity levels [21], suggesting that other itch mediators may also contribute to cholestatic itch.

Lysophosphatidic acid (LPA)—Recent study also identified LPA as a possible mediator for cholestasis-related itch [79]. The serum levels of LPA and autotaxin (ATX), the enzyme that converts lysophosphatidylcholine into LPA, are elevated in cholestatic patients with chronic itch. Notably, ATX levels are correlated with itch severity. Intradermal injection of LPA results in itching in mice [54;79]. LPA is a well known lipid mediator that can activate various cell types through the GPCRs LPA receptors (LPA₁₋₆) [27]. It is of great interest to test whether LPA can activate peripheral itch fibers via LPA receptors to produce itch.

PARs—Spicules of cowhage, when inserted into skin, produce a typical form of nonhistaminergic itch, via activation of cutaneous polymodal C-fibers in human [68;122]. Subsequent biochemical studies revealed that the active itch-inducing constituent is a cysteine protease named mucunain, which was shown to activate the protease-activated receptors (PARs), including PAR2 and PAR4 [122;135], by cleaving the extracellular N-terminus from PARs, allowing the N-terminus for being a ligand of its own receptor [135]. The signaling pathways downstream of PAR2 activation in sensory neurons and their role in itch are still incompletely understood. PAR2 couples to PLC β to sensitize TRPV1 and TRPV4 via activation of protein kinase C and A [9;10;31;48]. PAR2 activation also sensitizes TRPA1 [32] (Figure 1). In addition to DRG neurons, PARs are also expressed in non-neuronal cells in the skin [125;152]. Endogenous proteases such as trypsin, mast cell tryptase, kallikreins, and cathepsin S have been demonstrated to generate itch via activation of PARs [28;29]. In atopic dermatitis patients, the endogenous agonists and PAR2 per se increased in skin and PAR-2 agonists provoked enhanced and prolonged itch [143]. Consistently, over-expression of cathepsin S or PAR2 in the mouse skin causes dermatitis

and itching [41;73]. Thus, PARs especially PAR2 are key receptors to mediate acute and chronic itch via neuronal and non-neuronal mechanisms.

Mrgprs—Recent studies have revealed that several members of the Mrgpr family GPCRs, which are specifically expressed in subsets of small-sized DRG neurons, can serve as itch receptors [88;89;103;138] (Figure 1). Mrgprs family has more than 50 members in mouse and can be divided into several subfamilies: MrgprAs, MrgprBs, MrgprCs and MrgprD-G. However, there are only 10 members in human, and MrgprX1 is the mouse orthology of MrgprC11. Direct activation of MrgprA3 in a subset of primary sensory neurons by chloroquine, an antimalarial drug that can induce severe itch in human, induces itch in mice [88]. BAM8-22, an endogenous bovine adrenal medulla peptide induces itch through direct activation of MrgprC11 [88;138]. Activation of TRPA1 in DRG neurons is required for both MrgprA3 and MrgprC11-mediated and histamine-independent itch in mice [112;154]. In addition, α -alanine elicited histamine-independent itch through activation of a subset of MrgprD-expressing DRG neurons, which exclusively innervate the upper epidermis of the skin and are not-overlapped with the MrgprA3+ population [87]. The precise roles of Mrgprs and their endogenous agonists in chronic itch conditions are areas of interest.

TRP channels—Increasing evidence indicates that TRPV1 and TRPA1 ion channels are involved in both pain and itch. For example, capsaicin, the best known TRPV1 agonist, can elicit itching when applied, in a punctuate manner, to the epidermis [139]. Also, topically administrated capsaicin to normal human skin produces itch prior to burning sensation [22], suggesting that the superficial itch-mediating fibers express functional TRPV1. TRPV1 is also essential for mediating histamine-induced itch [136]. Furthermore, specific ablation of TRPV1-expressing neurons abolished both thermal pain and itch [62]. A regulatory subunit of TRPV1, Pirt (phosphoinositide binding protein), is required for both normal pain and itch [118]. TRPA1 agonists, such as AITC, allicin, and cinnamaldehyde, can elicit irritation in the skin, suggesting the role of TRPA1 in pain sensation [19]. TRPA1 is also required for Mrgprs-mediated and histamine-independent itch [112;148;154]. Our recent finding revealed that oxidative stress can also induce histamine-independent itch via activation of TRPA1, but not TRPV1 [92]. As oxidative stress is involved in various diseases associated with chronic itch, antioxidants and/or TRPA1 blockers may be used to treat chronic itch [92;112]. Although there is evidence suggesting that itch may be induced by activation of TRPV1 alone [139], TRP channels generally act as downstream effectors in pruriceptors for itch signaling transduction. Other TRP channels such as TRPC3 may also play a role for itch [148]. Thus, TRP channels represent molecular integrators for both pain and itch sensations.

Toll-like receptors (TLRs)—TLRs are typically expressed in immune cells to mediate innate immunity by recognition of pathogen-associated molecular patterns (PAMPs) [2]. Recently, several TLRs, such as TLR3, TLR4, and TLR7 have also shown to be functionally expressed in primary sensory neurons and play a role in detecting exogenous pathogens or endogenous danger signals [26;37;90;93;121]. We have demonstrated a novel role of TLRs in itch control [90;91;93]. Particularly, we identified TLR7 in a subset of primary sensory neurons that co-express TRPV1 and GRP [93]. TLR7 can recognize synthetic ligands such as imidazoquinoline derivatives (e.g., imiquimod and resiquimod) and guanine analogs (e.g., loxoribine) [55]. Intradermal application of TLR7 ligands induced dose-dependent scratching behavior in mice. Of note, imiquimod and loxoribine also elicit rapid inward currents and action potentials in capsaicin-sensitive DRG neurons, in a TLR7-dependent manner. This result suggests that the TLR7 ligands can directly activate and excite itch-mediating fibers. Furthermore, we found that TRPV1-expressing C fibers, but not TRPV1 per se, are required for imiquimod-elicited itch [93]. These findings suggest a possible coupling of TLR7 with ion channels in primary sensory neurons that can trigger itch

sensation. Notably, imiquimod also elicits TLR7-independent itch, which may be attributed to its off-target effects on adenosine receptors [133], inositol 1, 4, 5-trisphosphate receptors [74], and potassium channels [84]. Compared with wild-type (WT) mice, *Tlr7*^{-/-} mice exhibit normal thermal and mechanical pain. In contrast, *Tlr7*^{-/-} mice show a significant reduction in scratching behaviors in response to nonhistaminergic pruritogens, including chloroquine, endothelin-1, and SLIGRL peptide [93]. Thus, TLR7 may be involved in various forms of non-histaminergic itch.

We further observed TLR3 expression in small-sized primary sensory neurons that co-express TRPV1 and GRP [90]. The TLR3 agonist polyinosine-polycytidylic acid (poly(I:C)), a synthetic analog of double-strands (ds) RNA, also induces inward currents and action potentials in DRG neurons and elicits scratching in WT mice but not *Tlr3*^{-/-} mice [90]. Thus, TLR3 ligand may directly activate and excite itch-fibers to elicit itch. Unlike *Tlr7* deletion which only affects nonhistaminergic itch, deletion of *Tlr3* results in a significant reduction in scratching behaviors induced by both histamine-dependent and -independent pruritogens, in part due to additional actions of TLR3 in modulating spinal cord synaptic transmission and central sensitization (see below).

Are there itch-specific intracellular signaling pathways in nociceptors?

It was postulated that primary sensory neurons may be able to separate multiple sensations through distinct intracellular signaling [98]. Mounting evidence shows that primary sensory neurons that respond to pruritogens are also sensitive to other noxious stimuli, such as heat and pain [77;88;117]. For example, histamine-responding sensory neurons are also sensitive to capsaicin [136] and chloroquine-sensitive neurons also respond to capsaicin and mustard oil [154]. In addition, TRPV1-expressing primary sensory neurons are required for both thermal pain and itch [62]. It is possible that irritative chemicals may selectively activate distinct receptor and intracellular signaling pathways in the same neurons for transducing distinct itch or pain signaling. This hypothesis was supported by recent finding that SLIGRL peptide, which was regarded as a PAR2 agonist, can activate both MrgprC11 and PAR2 in DRG neurons: MrgprC11 mediates SLIGRL-induced itch, but PAR2 mediates SLIGRL-induced pain [53;89] (also see a prominent role of PAR2 for itch in the previous session). In addition, pruritogens may also activate different intracellular signaling pathways in sensory neurons. For example, Mrgprs are coupled to TRPA1 via distinct signaling: MrgprA3 is coupled to TRPA1 via G_{βγ} signaling while MrgprC11 can be coupled to TRPA1 via PLC signaling [154].

Are there itch-specific cutaneous sensory fibers?

It is widely accepted that primary sensory neurons that respond to pruritogens are also sensitive to pain stimuli. Thus, it was doubted whether there are itch-specific neurons. However, continuous attempts have been made to identify itch-specific neurons. Of note, some mediators important for pain sensation and development of nociceptors, such as galanin, are not required for itch sensation [56]. Also, various pruritogens, such as histamine, chloroquine, and BAM8-22, were shown to activate a subpopulation of nociceptors [103]. Furthermore, Schmelz and collaborators identified a population of mechanical-insensitive C-fibers that are responsive to histamine, and the firing of these C-fibers matched the itch sensation in human [132], suggesting that these C-fibers might be specific for histamine-induced itch. Capsaicin, a highly potent algescic compound, is able to induce itch in human when specifically delivered to the epidermis [139], suggesting that a subpopulation of TRPV1-expressing fibers that exclusively innervate the superficial layers of the epidermis could be itch-specific.

Recently, Dong's group, in collaboration with LaMotte's group, has identified MrgprA3-expressing neurons as itch-specific neurons in mice [51]. MrgprA3 defines a unique population of DRG neurons that co-express calcitonin gene-related peptide (CGRP) but not substance P. In addition, MrgprA3 is also co-localized with IB4 (nonpeptidergic marker) and TRPV1. Interestingly, MrgprA3+ nerve fibers exclusively innervate the epidermis of the skin, providing an anatomical basis for skin-elicited itch. Ablation of MrgprA3-expressing neurons in mice attenuated itch, induced by various pruritogens such as histamine, chloroquine, SLIGRL, BAM8-22, 5-HT, and ET-1, except α -alanine. Moreover, chronic itch induced by dry skin and allergic responses was also decreased in these mice lacking MrgprA3-expressing neurons. Consistently, ablation of CGRP+ population abolished histamine and chloroquine-induced itch [102]. To determine whether selective activation of MrgprA3-expressing fibers by capsaicin would induce itch, Dong's group employed an elegant strategy to re-express TRPV1 exclusively in MrgprA3-expressing neurons in TRPV1 knockout mice. Strikingly, intradermal injection of capsaicin into the cheek of these mice only induced itch-indicative hindlimb scratching behavior but not pain-indicative forelimb wiping behavior. Thus, selective activation of itch-specific neurons (MrgprA3) only induces itch, regardless the stimulus modality.

Central mechanisms of itch

Spinal cord mechanisms of itch

The itch-specific neuronal circuitry in the spinal cord has also begun to be revealed. Recent studies from Zhen's group highlighted an essential role of the GRP/GRP receptor (GRPR)-expressing neurons in the spinal cord for itch transmission [94;144;145]. GRPR is expressed in superficial dorsal horn neurons in the laminae I and II [144]. GRPR mutant mice display reduced scratching induced by multiple pruritic agents, especially nonhistaminergic pruritogens, whereas pain sensation is intact in the mutants [144]. Importantly, ablation of GRPR+ neurons in the dorsal horn by intrathecal injection of the neurotoxin bombesin-saporin markedly reduced scratching, evoked by both histaminergic and nonhistaminergic pruritogens [145]. In contrast, elimination of GRPR+ neurons had no effects on pain behaviors. Thus, GRPR+ neurons define a specific population for mediating itch [145]. A follow-up study shows that intrathecal morphine can also activate GRPR to elicit itch by binding to the μ -opioid receptor (MOR) isoform MOR1D that is cross-linked to GRPR [94]. Intriguingly, MrgprA3+ primary afferents may form direct synaptic connections with GRPR+ spinal cord neurons [51]. Together, peripheral itch-specific fibers may directly project to the itch-mediating neurons in spinal cord.

Although GRP peptide was detected in a subset of DRG neurons [90;144], the role of DRG neurons-derived GRP in itch transmission is still unclear. It was found that GRP is more prominently expressed in spinal cord neurons within laminae I and II [40]. It has also recently been demonstrated: 1) capsaicin induced CGRP but not GRP release; and 2) preincubation of capsaicin to deplete neuropeptides from primary afferents prevented bile acids-evoked CGRP release but not GRP release in spinal cord slices [8]. This is consistent with an early observation that capsaicin induced SP but not GRP release in spinal cord slices, while depolarization with KCl evoked Ca^{2+} -dependent GRP release [108]. Thus, activation of peripheral TGR5-expressing itch receptors by bile acids might release GRP from spinal neurons but not from primary afferents. Although glutamate was proposed as the principal excitatory neurotransmitter between C fibers and GRPR+ spinal neurons [78], SP and CGRP may also play a role in itch transmission in the spinal cord. In addition to a central role of GRP in itch control, serum levels of GRP were found to be correlated with itch severity in atopic dermatitis patients [69], and intradermal injection of GRP induced scratching behavior in mice through activation of mast cells [13]. Therefore, GRP also plays a role in peripheral itch signaling.

The population of the SP receptor NK1-expressing neurons, most of which are spinothalamic tract (STT) neurons, has been implicated in both pain and itch sensation [61]. In rats, elimination of NK1R+ neurons with intrathecal substance P-saporin in the spinal cord leads to deficits in both pain and itch, suggesting that this cell population of neurons is critical for both itch and pain [25;101;111]. However, the precise role of NK1+ neurons in mediating itch in mice still need further investigation. It will be of particular interest to examine whether NK1+ neurons and GRPR+ neurons are overlapping (Box-2).

A subset of spinal inhibitory interneurons, whose development requires the expression of the transcription factor Bhlhb5, was recently identified to be required for itch suppression [127;128]. Ablation of Bhlhb5 from an inhibitory interneuron population, which can also be labeled by the transcription factor Pax2, leads to skin lesions and enhanced scratching in mice [127]. Future studies are needed to determine the relationship between Bhlhb5+ neurons and GRPR+ neurons and the neurotransmitters that are used by Bhlhb5+ itch-suppressing neurons (Figure 2 and Box-2).

We recently identified TLR3 as a critical regulator of synaptic transmission and central sensitization underlying itch induction and sensitization in mice [90]. TLR3 is not only expressed in cell bodies of DRG neurons but is also transported to central axonal terminals in the spinal cord to modulate synaptic transmission in capsaicin-sensitive C-fibers [90]. Deletion of *Tlr3* results in a reduction in excitatory synaptic transmission (i.e. decrease in the frequency of spontaneous excitatory postsynaptic currents, sEPSCs). Induction of long-term potentiation (LTP) in the intact spinal cord is also abolished in *Tlr3*^{-/-} mice but not *Tlr7*^{-/-} mice [90]. Thus, TLR3 may regulate itch via both peripheral and central mechanisms [90].

Brain Mechanisms of itch

Brain imaging techniques, such as positron emission tomography (PET) and functional magnetic resonance imaging or functional MRI (fMRI), have begun to unravel the brain circuitries for itch perception [61;153]. Itch is a multidimensional perception encompassing sensory discriminative, affective, and emotional components. It is extensively processed by the brain network, including the primary and secondary sensory cortex, supramarginal gyrus, inferior parietal lobe, anterior and posterior cingulate cortex, precuneus and insula-claustrum complex [72]. Although itch and pain are distinct sensations, the same brain regions, such as the amygdala, hippocampus, and hypothalamus, can be activated by both sensations. In healthy volunteers, histamine-induced itch activates a diffuse brain network, while cowhage-induced histamine-independent itch induces greater activation of the insula, thalamus, and putamen [120].

Mechanisms of chronic itch

Peripheral sensitization and neural-immune interactions

Skin injury and inflammation result in recruitment of immune cells (e.g., diverse innate immune cells, T lymphocytes). Activated immune cells release proinflammatory mediators to sensitize pruriceptors. This peripheral sensitization contributes importantly not only to chronic pain and but also to chronic itch [18;61]. In pathological conditions, the population of pruriceptors could be enlarged and also exhibits enhanced responses to pruritogens [3;5]. For example, in dry skin-induced chronic itch, the populations of primary sensory neurons responding to PAR2 agonist and 5-HT are enlarged, which is associated with enhanced scratching responses to these pruritogens [3]. In the skin, neural-mast cell and neural-keratinocyte interactions play an important role in peripheral sensitization [61;98]. Degranulation of mast cells leads to the release of histamine [30;38;136], serotonin [71;156], tryptase[143], TNF- [157], prostaglandin E1/2 (PGE1/2) [97] and leukotriene B4

(LTB4) [11;12;14]. Activated keratinocytes also release ET-1, proteases (e.g. cathepsin S) [123] and β -endorphin [82;83;158]. These inflammatory mediators sensitize pruriceptors or/and disrupt barrier function in the epidermis and result in dry skin injury [61]. T cells can release cytokines such as IL-2 and IL-31, which have been shown to activate and/or sensitize pruriceptors. In addition, neurotrophins such as nerve growth factor (NGF) from keratinocytes or mast cells [129] and artemin from fibroblasts [109] may cause long-term structural reorganization (sprouting) of free nerve endings including itch fibers (Figure 3). In atopic dermatitis, contact dermatitis and prurigo nodularis, serum and local NGF is remarkably increased [59]. Interestingly, NGF up-regulation in dry skin is reduced in *Tlr3*^{-/-} mice, which is associated with a substantial reduction of chronic itch in these knockout mice [90]. Anti-inflammation and anti-NGF strategies may be beneficial for treating both chronic itch and chronic pain.

Cytokines, including IL-2, IL-6 and IL-31, are recently highlighted as key mediators for itch sensation [142] (Figures 1 and 3). Among them, IL-31 is getting more attention. Transgenic mice over-expressing IL-31 developed chronic itch, alopecia, and skin lesions [36;141]. The activated T lymphocytes, particularly T helper type 2 cells, are one of the main sources for IL-31. IL-31 binds to a heterodimeric receptor composed of IL-31 receptor (IL-31RA) and oncostatin M receptor (OSMR) to activate several signaling pathways, such as the Janus tyrosine kinase (JAK), signal transducers and activators of transcription (STAT), phosphatidylinositol 3-kinase/AKT (PI3K/AKT) and p38 MAPK pathway in keratinocytes and monocytes [36;141]. Fluorescence activated cell sorting (FACS) analysis also showed that the skin of chronic atopic dermatitis patients contains increased levels of IL-31-producing T cells. IL-31 mRNA is significantly up-regulated in serum and skin of patients with pruritic atopic dermatitis [39;141;147]. Canine IL-31 was also detected in the majority of dogs with naturally occurring atopic dermatitis and intradermal injection of it induced pruritic behaviors in dogs [46]. Higher levels of blood β -endorphin and IL-31 significantly correlated itch severity in atopic dermatitis patients [83]. Moreover, immunohistochemistry data showed that IL-31RA and β -endorphin were increased and colocalized in the skin of atopic dermatitis patients [83]. IL-31 receptor activation in keratinocytes induces calcium influx and STAT3-dependent production of β -endorphin, leading to itch this is associated with atopic dermatitis [83]. Application of IL-31 antibody reduces scratching behavior in NC/Nga mice with atopic dermatitis [50]. IL-31RA and OSMR are co-expressed in a subset of DRG neurons [16], suggesting that T cell-derived IL-31 may directly excite primary sensory neurons to elicit itch sensation. However, the antibody used for the localization of IL-31RA in this paper also showed positive stains in IL-31RA KO mice [16], raising the concern of non-specific staining. Thus, the direct evidence for the expression of IL-31RA in DRG neurons and activation of DRG neurons by IL-31 is still lacking. Nevertheless, IL-31 may serve as an ideal mediator for neural-immune interaction in chronic itch.

Central sensitization and neural-glia interactions

In chronic pain, tissue injury and persistent noxious input to the spinal cord result in central sensitization [64], i.e. hyperactivity of spinal cord projection neurons and excitatory interneurons, which is responsible for allodynia (pain induced by normally innocuous stimuli, e.g., light touch), and hyperalgesia (enhanced responses to noxious stimuli, e.g. pinch) [18]. In chronic itch, ongoing activity of pruriceptors after skin injury also results in central sensitization to drive itch. Enhanced responses of spinal cord neurons to PAR-2 agonists were demonstrated in a dry skin model [5]. Peripheral GRP expression was up-regulated in a mouse model of pruritic atopic dermatitis [149]. GRP/GRPR signaling in the spinal cord may also be up-regulated in chronic itch to enhance itch-related central sensitization.

It is well established that activation of glial cells in the spinal cord plays an important role in the pathogenesis of chronic pain [23;42;124]. Microglia and astrocytes are activated following tissue or nerve injury [66;67]. Activation of p38 MAPK in microglia results in the expression and release of the cytokines TNF- α and IL-1 β and the brain-derived neurotrophic factor (BDNF) [65]. On the other hand, activation of c-Jun N-terminal kinase (JNK) signaling in astrocytes results in the release of chemokine (C-C motif) ligand 2 (CCL2) [44]. These glial mediators (TNF- α , IL-1 β , BDNF, and CCL2) can cause central sensitization and persistent pain by enhancing excitatory synaptic transmission and suppressing inhibitory synaptic transmission in spinal cord neurons [43;44]. Whether these glial mediators can cause central sensitization of GRPR-expressing pruriceptive neurons in the spinal cord warrants further investigation. Glial activation may also be involved in neuropathic itch [114;115]. Intramedullary cavernous hemangiomas was associated with chronic neuropathic itch in the corresponding dermatome and featured as gliosis and hemosiderin deposition after hemorrhage [24;35;81].

Central sensitization in the brain regions is also involved in chronic itch. Allergen-induced itch in patients provokes robust activation of the striato-thalamo-cortical circuit, which is involved in motivational processing and associated with the urge to scratch in chronic itch condition [85]. In addition, descending facilitation from the cortex/brain stem to the spinal cord, which has been strongly implicated in chronic pain sensitization, may also play a role in chronic itch [72].

Crosstalk between the itch and pain pathways

Crosstalk between the itch and pain pathways in physiological conditions

Itch and pain represent two distinct unpleasant sensations and evoke distinct behavioral responses: pain evokes withdrawal reflex while itch elicits scratching responses [61]. Itch and pain are also closely related: itch can be suppressed by scratching and painful stimuli, such as noxious heat, noxious chemicals, and electric shock in human [131]. In line of these observations, itch is also inhibited within the area that can generate capsaicin-induced secondary mechanical hypersensitivity in human [47]. Interestingly, scratching behaviors induced by chloroquine [49], imiquimod [74;93] and hydrogen peroxide [92] display inverted U-shaped dose-response curves in rodents, suggesting that these pruritogens at higher doses produce pain to suppress itch. It was also found that mouse strains that are sensitive to pain are less sensitive to itch and vice versa [49].

On the other hand, inhibition of pain may produce itch [61]. The best-known example is spinal administration of morphine, which inhibits pain but elicits itch [107]. It was found that spinal itch-responding neurons do not exhibit spontaneous activity, possibly due to the tonic inhibition of these neurons by pain-relay neurons that are spontaneously active [61;119]. It was speculated that morphine-induced itch may be attributable to disinhibition of itch, by relieving pain-induced suppression in the spinal cord [15]. However, this hypothesis was challenged by a recent study showing that intrathecal morphine produces itch via direct activation of the MOR isoform MOR1D that forms a heterodimerizer with GRPR, whereas morphine-induced spinal analgesia is mediated by a different isoform, MOR1 [94]. Thus, morphine may directly activate itch-selective neurons in spinal cord to produce itch, and therefore, morphine-induced analgesia is not a prerequisite for the generation of itch. However, it is still important to investigate whether inhibition of pain is sufficient for the induction of itch.

The population coding theory was proposed to explain the coding of pain versus itch and has gained experimental support [7;96;99;100;117]. As demonstrated in Figure 2, there are two populations of primary sensory neurons that respond to pain and itch, respectively. Itch-

specific population (pruriceptors) such as MrgprA3⁺ neurons is a much smaller population within pain-specific population (nociceptors) expressing TRPV1. Notably, pruriceptors are polymodal and also respond to algogens such as capsaicin or mustard oil [88;89;144;145]. Activation of itch afferents stimulates release of certain neurotransmitters from the spinal cord central terminals of primary afferents, leading to subsequent release of GRP and opioid peptide(s) to activate itch-selective neurons that express GRPR and MOR1D. If a stimulus selectively activates the small itch-population, regardless of the stimulus modalities, it could generate itch. On the other hand, activation of a larger population of pain primary afferents will release glutamate and neuropeptides (SP and CGRP) in the spinal cord to activate pain-mediating neurons (e.g., NK1R⁺ neurons), and meanwhile, also activates Bhlhb5⁺ inhibitory neurons to suppress itch. If a strong stimulus activates both pain and itch afferents, pain would dominate by masking itch via an inhibitory circuit (Figure 2). Mixed pain and itch sensations may also be produced, if pain stimulus is not sufficient to mask itch [99].

Crosstalk between the itch and pain pathways in pathological conditions

Recent animal studies suggested that dysfunction of pain-itch crosstalk may be involved in the development of chronic itch [95;127] (Figure 3). Mice lacking a subset of inhibitory interneurons, labeled by the transcription factor Bhlhb5 in spinal cord dorsal horn, display enhanced chronic itch phenotype [127]. Another study showed that ablation of vesicular glutamate transporter 2 (VGLUT2)-dependent glutamate release from Nav1.8-expressing nociceptors leads to pain deficits and itch hypersensitivity in mice [95]. Notably, intradermal injection of capsaicin induces itch but not pain in these conditional knockout mice lacking *vGlut2* in nociceptors [95]. It is suggested that glutamate released from the central branches of nociceptors not only triggers pain transmission but also activates inhibitory neurons for itch suppression.

Under the pathological conditions, painful stimuli may not be sufficient to suppress itch. Instead, pain may enhance itch in chronic itch patients. Itch stimuli can be perceived as pain in some chronic neuropathic pain condition [17]. For example, normally painful stimuli, such as electrical stimuli, bradykinin or acetylcholine evoked itching in patients with atopic dermatitis [57;113] and mice with inflamed skin [86]. It is noteworthy that in “itchy skin”, touch or brush evokes itch near the site of itch, termed “alloknesis” [6].

Concluding remarks

Although closely related to pain, itch is a distinct sensation that causes scratching. Recent progress has indicated the existence of an itch-specific neuronal circuitry, formed in part by the MrgprA3-expressing primary sensory neurons and GRPR-expressing neurons in the superficial spinal cord. There is distinct crosstalk of itch and pain in physiological and pathological conditions. Acute itch can be inhibited by scratching and painful stimuli. An antagonistic interaction between pain and itch is further revealed by morphine-elicited itch and analgesia. Itch and pain employ largely overlapping receptors, such as TRPV1, TRPA1, TLRs and PARs.

Itch and pain appear to have more similarities in pathological and chronic conditions (Box-2). Dysfunction of the pain-itch crosstalk in pathological conditions may trigger chronic itch. Central sensitization, which has been widely implicated in the genesis of chronic pain (e.g., touch-evoked pain, allodynia), also play an important role in chronic itch, as manifested by and touch-evoked itch (alloknesis). Chronic itch is often diagnosed and treated by dermatologists as an immune disease. However, chronic itch is also a disorder of the nervous system, triggered by immune dysfunction. The striking similarities between chronic itch and chronic pain have important clinical implications, because chronic pain and

chronic itch are likely to coexist in patients, such as those with neuropathic postherpetic pain and itch [114;115]. Many treatments and prescribed drugs for chronic pain control are also effective for treating chronic itch. For example, treatments like vagus nerve stimulation [76] and acupuncture [110] and drugs like gabapentin and pregabalin [1;61;140] have been shown to reduce chronic itch after burn, renal failure and multiple sclerosis. Antidepressants, such as amitriptyline and mirtazapine, which are widely used for treating neuropathic pain, also reduced psychiatric itch and cholestasis itch [61].

There are many outstanding questions remaining in the itch field, as indicated in Box 2. The neurotransmitters for spinal cord itch transmission and the identities (projection neurons vs. interneurons) of GRPR-expressing neurons are still unclear. It is also of great interest to investigate whether and how glial cells control itch transmission. Do cytokines play distinct role in regulating pain and itch transmission? Notably, the currently used animal models focus on acute itch induced by individual pruritogens or chronic itch evoked by atopic dermatitis and dry skin injury. However, animal models are still unavailable to mimic chronic itch after systemic diseases, such as liver or kidney diseases. The development of clinically relevant animal models of chronic itch is urgently needed. Little is known about the brain modulation of itch. Brain-imaging techniques and psychophysical protocols have been developed to study itch in humans, which will help to clarify the brain mechanisms of chronic itch and further reveal the interactions of itch and pain in the brain. Despite the fact that drugs for chronic pain treatments have demonstrated some success in chronic itch relief, elucidation of unique mechanisms underlying chronic itch will eventually lead to more selective anti-itch treatments.

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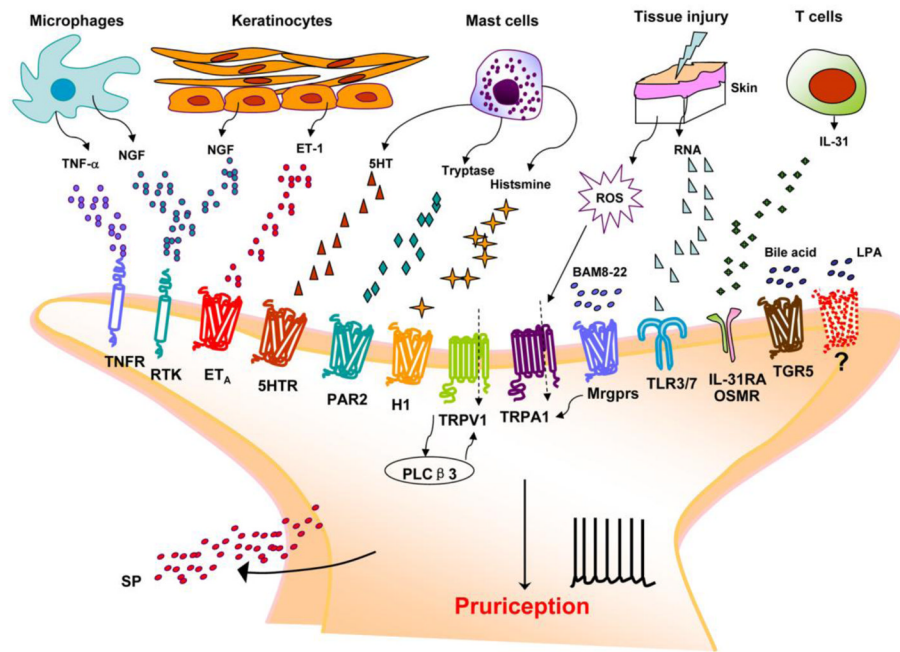


Figure 1. Peripheral itch mediators and their respective receptors in skin nerve terminal
 Upon tissue damage or inflammation, skin cells (keratinocytes, fibroblasts) and residential and infiltrating immune cells (macrophages, mast cells, neutrophils) release itch mediators that can activate pruriceptors in nerve terminal of C-fiber nociceptor. These itch mediators (pruritogens), such as ET-1, serotonin, histamine, tryptase, thromboxanes, leukotrienes, bile acids, LPA, nerve growth factor (NGF), tumor necrosis factor (TNF-) and RNAs can directly bind their cognate receptors such as GPCRs (ETA, 5HTR, H1R or H4R, PAR2/4, Mrgprs, LPAR, TGR5), TLRs (TLR3/7), and cytokines receptors (IL-31R/OSMR), expressed by pruriceptors. Activation of these itch receptors results in activation of PLC β 3 and increases in intracellular calcium through TRPV1 and/or TRPA1 to elicit itch transduction and sensation (pruriception). Activation of pruriceptors also releases additional itch mediators such as substance P to induce neurogenic inflammation and potentiate itch signaling.

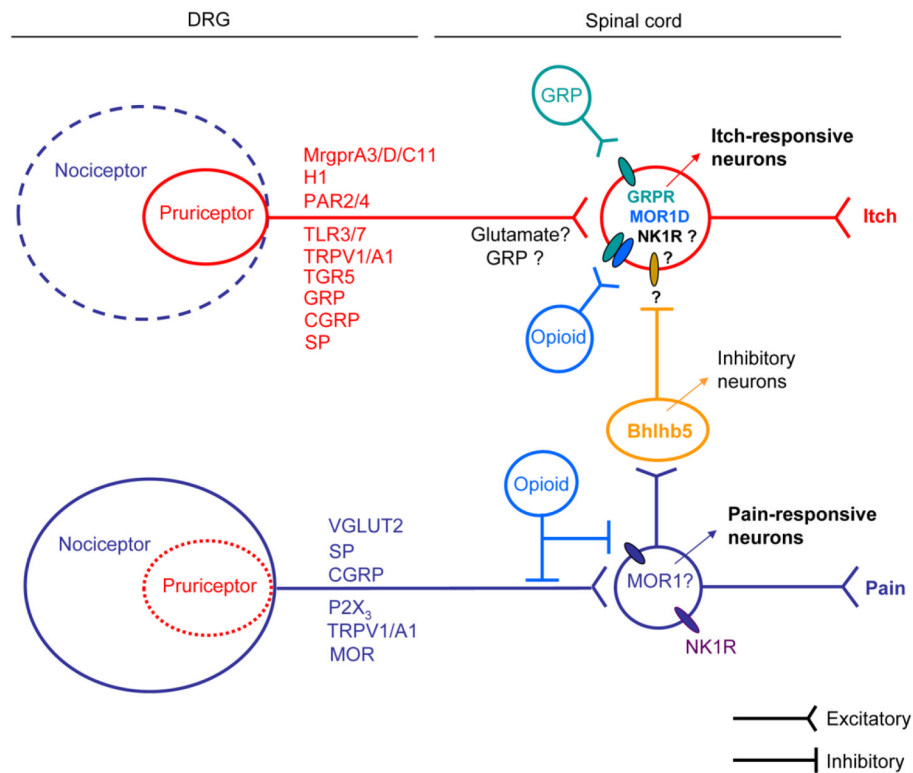


Figure 2. Crosstalk of the itch and pain pathways in physiological conditions

Itch and pain are encoded by the unique labeled lines in the physiological conditions. In dorsal root ganglia or trigeminal ganglia, the large population of pain-responsive neurons (e.g., TRPV1-expressing neurons) consists of the small population of itch-responsive neurons (e.g., MrgprA3-expressing neurons) which are polymodal and also respond to painful stimuli. These pruriceptors may also express H1, PAR2, TLR3/7, TRPA1/V1, and neuropeptides (GRP, CGRP, SP). Activation of itch primary afferents stimulates neurotransmitter release from the central terminals of the pruriceptors in the spinal cord dorsa horn, leading to GRP and opioids release to activate GRPR and MOR1D-expressing neurons for itch transmission. Activation of nociceptors results in the activation of secondary nociceptive neurons (e.g., NK1R+ neurons) in the spinal cord for nociceptive transmission. Activation of nociceptors also causes subsequent activation of Bhlhb5+ inhibitory neurons to suppress itch transmission in GRPR+ neurons. In addition, spinal cord opioids can activate MOR1 to suppress pain and also activate MOR1D to elicit itch via its coupling to GRPR.

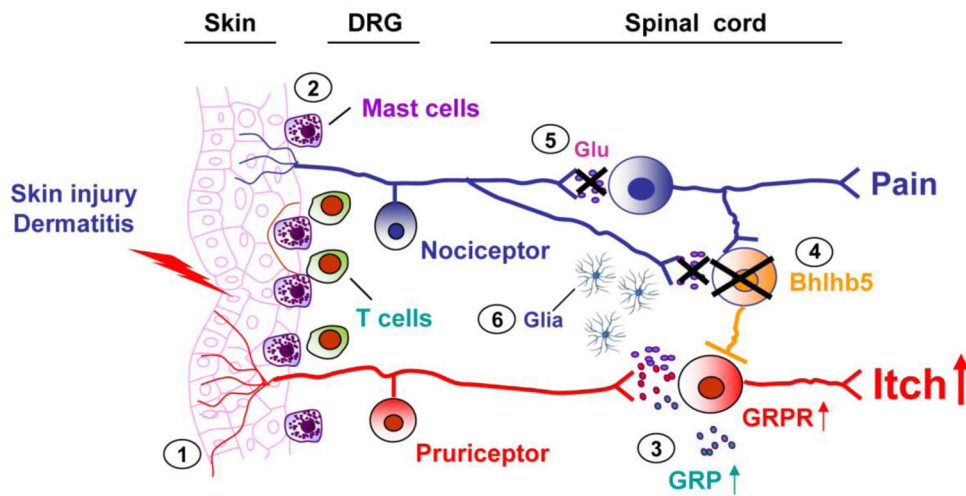


Figure 3. Crosstalk of the itch and pain pathways in pathological conditions

In pathological conditions, multiple mechanisms contribute to the development of chronic itch. (1) Activation of mast cells and T cells in injured skins causes the release of itch mediators such as histamine and IL-31, leading to the activation of pruriteptors and induction of peripheral sensitization. (2) Growth factors (e.g., NGF or artemin), released from skin cells may cause sprouting of C-fibers (including pruriceptive fibers), leading to itch hypersensitivity. Furthermore, tissue injury and damage or dysfunction of the nervous system causes central sensitization in spinal cord neurons which is associated with (3) up-regulation of GRP and GRPR, (4) decrease or loss of inhibitory control from Bhlhb5+ neurons, (5) decrease of VGLUT2-dependent glutamate release from nociceptors, and (6) activation of glial cells (astrocytes or microglia) in the spinal cord. Thus, the crosstalk of the pain and itch labeled lines are disrupted in pathological conditions. Central sensitization not only drives chronic pain but also potentiates and maintains chronic itch. In addition, descending facilitation from the brainstem may further potentiate chronic itch. These mechanisms of peripheral and central sensitization are also known to drive chronic pain.