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Mrgprs: An Essential Role In Itch

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Abstract

Itch is an unpleasant cutaneous sensation associated with a desire to scratch. It is not merely as a clinical symptom of a variety of diseases but also a disease. Itch can be elicited by a variety of endogenous pruritogen molecules and exogenous substances that activate the membrane receptor and ion channel of neurons. Looking for and identification novel itch related receptors are essential to understand the physiological and pathological mechanism of itch. Mas-related G-protein-coupled receptors (Mrgprs) have been discovered about more than 10 year which are exclusively distributed in the peripheral nervous system and relational tissues. They play a special role in the evoked and transmission of itch. Pruritogen such as chloroquine, small bovine adrenal medulla 8-22 (BAM 8-22) peptide, SLIGRL and β-alanine can activate the member of Mrgprs family to induce scratching. In this paper, we will review the discovery, distribution and function of Mrgprs in acute and chronic itch.

Introduction

Organisms need to sense and respond to the change in the internal system and external environment in order to survive or avoid a harmful situation. They perceive stimulation and receive inputs from internal and external environment dependent on the sensory system which included the itch sensation. A variety of exogenous and endogenous pruritogen including small peptides, cytokines, lipids, proteases and amine can evoke obviously scratching behavior [1]. Receptors and channels expressed on the peripheral nervous system nerve fiber endings are capable of sensing itch stimulation and transduction itch signals to the central nervous system [2,3]. The family of G protein-coupled receptor is research focus which is the most diverse proteins and participates in many physiological functions [3]. G protein-coupled receptors (GPCRs) are also engaged in itching sense detecting and transmission [4-6].

In recent years, there has been a novel class of G protein-coupled receptor (Mas-related G protein-coupled receptors, Mrgprs), which specifically express in the peripheral nervous system or relative tissues. Some of them are involved in mediated of itch such as MrgprA3, MrgprC11, MrgprD, MrgprB2. Scratching behavior which was induced by activated Mrgprs is not histamine-dependent [7-10]. Therefore, the role of Mrgprs in itch is of profound significance to understand pruritus deeply and the development of antipruritic drugs. This review summarizes our current state of knowledge of the Mrgprs in itch, with particular emphasis on its physiological function.

Mrgprs Overview

Since the capsaicin receptor (TRPV1) has been cloned in mammals for the first time in 1997 and it is related to pain. After that, other TRP channels such as TRPA1 and TRPM8 were discovered one after another. These exciting discoveries have been promoting the researchers to discovery more sensory specificity molecules, which also include pruritogen [11-14]. Mrgprs family was discovered in 2001 which are especially for peripheral sensory transmission [15]. The Mrgprs family was GPCR which was 35% sequence homology with the proto oncogene Mas1 called Mas-related genes (mrgs) [16]. and Mas1 is the putative receptor for peptide angiotensin[1-7]. which was involved in blood pressure and control of osmolarity [17]. Therefore, Mrgprs was named Mas1-related G protein-coupled receptor. Mrgprs comprises about 50 members of the gene and pseudogenes in total in the mice. Human Mrgprs was less than the mice, of which 18 genes were found only [15]. Some of their functions have already been made clear, however, most of them are not yet known at present.

Identification of the Mrgprs

Two research groups identified Mrgprs with different methods independently [15,18]. A novel cDNA clone was identified as Mrgprs genes which encode nociceptive GPCR by using a cDNA subtractive screening performing in wild-type and Ngn1$^{-/-}$ mice. Another research group in Canada, Lembo and his colleagues discovered and identified seven Mrgprs from rats and humans by

degenerate PCR in 2002. These Mrgprs are restricted expressed in highly specialized neuronal population, the small-diameter dorsal root ganglion (DRG) and the trigeminal ganglion (TG) neurons which were named as sensory neuron-specific G protein-coupled receptors (SNSRs) [18]. SNSRs is the member of Mrgprs family and is homologous to some members of the Mrgpr family, but they have different nomenclature. For example, hSNSR and hMrgprX3 are the same GPCR [18-20].

Express of the Mrgprs

Mrgprs gene is involved in human genome, which was also discovered in other species, including rodent and primates such as mouse, rats, gerbils, macaques [18-21]. Rodents and human Mrgprs are divided into different subpopulation according to orthologs. Mrgprs of mouse and rat include: MrgprAs (mMrgprA 1-22 and rMrgprA), MrgprBs (mMrgprB1-13 and rMrgprB1-10), MrgprCs (mMrgprC1-14 and rMrgprC). In addition, mouse and rat Mrgprs also include six single-member genes subfamily which have obvious human orthologs. In primates, seven Mrgprs were called rMrgprA X1-7 [15,22]. Together, Mrgprs is a great family comprising more than 50 member [15,22]. In rodents, there is a clear Mrgprs orthodox among populations, but the rodents Mrgprs gene is lowly homologous with humans Mrgprs gene. Which may lead to findings based animal model in clinical applications will encounter some risks and confusing (Figure 1).

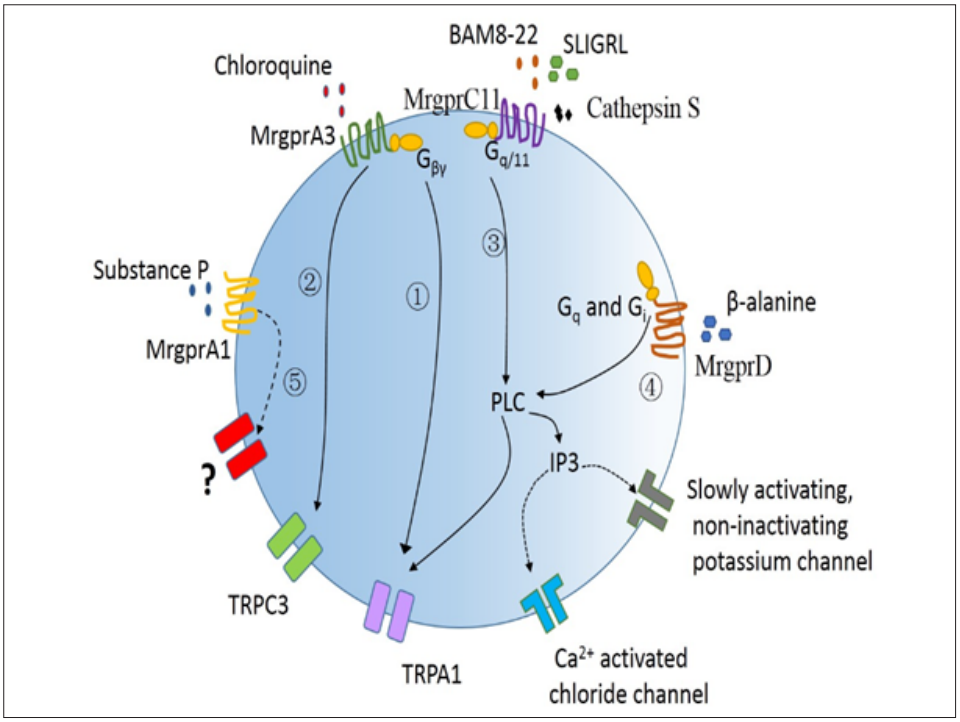

Figure 1: Signaling Pathways of Mrgprs Response on Drg Neurons Activated By Itch Substances.

The role of Mrgprs in itch

MrgprA3

Distribution and expression of MrgprA3

The location of MrgprA3 gene varies among different species. In mice, the gene of MrgprA3 was located on chromosome 7 and encoded a member of the large G-protein-associated receptor family. Similarly, the rats MrgprA3 gene was found on the 1 chromosome. Likewise, the humans MrgprA3 gene was found on 11 chromosome respectively [15,22]. The MrgprA3 gene was clustered in the genome with other Mrgprs such as MrgprA1, MrgprC11. According to cluster characteristic of Mrgprs, a Cluster-knock out mouse (Mrgpr-cluster $\Delta^{-/-}$ mice) was generated by the 12 genes in Mrgprs were knocked out, which also includes MrgprA3. Mrgpr-cluster $\Delta^{-/-}$ mice was used in acute itch model research and contribute to finding that MrgprA3 involved in the mechanism of chloroquine mediated itch [7].

Most of the Mrgprs including MrgprA3 are exclusively expressed on small diameter peripheral DRG and TG sensory neurons excluding a few Mrgprs (such as MrgprB2 and MrgprX1) which expressed on mast cell [22]. MrgprA3$^+$ neuron project to dorsal horn lamina of spinal cord connected with GRPR$^+$ neurons and MrgprA3$^+$ fiber present at the peripheral skin. MrgprA3$^+$ nerve fibers exclusively innervate the epidermis of skin, which does not express on other organs and tissues such as lung, heart, stomach [9]. MrgprA3$^+$ fibers are denser extent in hairy skin than glabrous skin. MrgprA3$^+$ nerve fibers were seen occasionally wound hair follicle in mice skin [9]. Studies also have shown that MrgprA3$^+$ nerve fibers express in the skin anywhere included head, trunk of the mouse's body and limbs. The epidermal MrgprA3$^+$ nerve fiber density is higher in the skin of back, face, limbs and soles, lower in the skin of abdomen and neck [23]. In the spinal cord, MrgprA3$^+$ nerve fibers form synaptic connection with 67% gastrin releasing peptide receptor (GRPR) positive neurons which can be activated

by pruritogen gastrin release peptide, suggesting that MrgprA3 is closely related to the itch which only express on 5% of all DRG neurons. MrgprA3$^+$ fibers are the slower conduction velocity C fiber that responded to capsaicin, mechanical force and heat noxious stimuli. Furthermore, distribution of positive MrgprA3 neurons was not the same in different segments DRG in *Mrgpra3GFP-Cre; ROSA26tdTomato* mice. It was found that MrgprA3$^+$ neurons appear in DRG of thoracic, lumbar and caudal segments, mostly a class of small diameter neurons. The density of MrgprA3 positive neurons was high in DRG of the thoracic and lumbar and was low in DRG of tail segment [23].

Property of MrgprA3$^+$ neurons

MrgprA3$^+$ neurons are mainly nonpeptidergic neurons that labeled by nonpeptidergic marker. However, parts of MrgprA3$^+$ neurons were labeled by both peptidergic and nonpeptidergic marker. 79.1% MrgprA3$^+$ neurons expressed IB4 which is a nonpeptidergic marker and do not express substance P (SP), but 84.2% MrgprA3$^+$ neurons expressed another peptide marker CGRP, 63.1% MrgprA3$^+$ neurons express both IB4 and CGRP [9,22]. GPCRs and ion channels are the components of GPCRs-ion channel axis that exerts its function of transmission pain and itch. TRP channels of sensory nerves in peripheral are typical ionic channel which sense endogenous and exogenous chemical, mechanical, and thermal stimuli including TRPV1 and TRPA1 play a role in itching. MrgprA3$^+$ neurons expressed both TRPV1 and TRPA1, 88.3% of MrgprA3$^+$ neurons were TRPV1 positive cell, Only 43% of MrgprA3$^+$ neurons were TRPA1$^+$ [9,23]. TRPC3 and TRPM8 are additional ionic channel present on the MrgprA3$^+$ neuron [24]. In addition to the TRP channels, MrgprA3$^+$ fiber in the skin also express calcium-activated chloride channels [25]. Moreover, other receptors of the Mrgprs family were also of observed on MrgprA3$^+$ neurons, 93.3% of MrgprA3$^+$ neurons expressed MrgprC11.

Action potentials-related parameters of MrgprA3$^+$ neurons were significantly different from MrgprA3$^-$ neurons. The action potentials overshoot, maximum rise slope (10–90%) of MrgprA3$^+$ neurons were significantly larger than the MrgprA3$^-$ neurons. Furthermore, after potential decay duration of MrgprA3$^+$ neurons were longer than MrgprA3$^-$ neurons [23]. The rest membrane potential of MrgprA3$^-$ neurons is -63 mV, however rest membrane potential of MrgprA3$^+$ neurons was lower than MrgprA3$^-$ neuron, which is -53--55mV [23]. Current threshold of MrgprA3$^+$ neurons was higher and most of MrgprA3$^+$ neurons cannot induce repeated action potentials, which only induced usually a single action potential by injecting a strong depolarizing current. But repeated firing of MrgprA3$^-$ neurons were induced by injecting strong current [23]. Sodium and potassium channels contributed to the repeated firing, which modulated duration of depolarization phase and after hyper polarization phase. Tang showed that potassium current difference was observed between MrgprA3$^+$ and MrgprA3$^-$ neurons that MrgprA3$^+$ neurons showed a relatively greater potassium current than MrgprA3$^-$ neuron [23]. The reason why MrgprA3$^+$ neuron cannot be duplicated is that: 1) the resting potential of MrgprA3$^+$ neurons is lower than that MrgprA3$^-$ neuron (-63mV), far from the threshold; 2) after potential of MrgprA3$^+$ neurons, which cannot induced action potential burst, is longer that of MrgprA3$^-$ neurons; 3) The decrease in excitability of MrgprA3$^+$ neurons is due to more potassium currents in these neurons. This will mean that the MrgprA3$^+$ neurons produce an action potential that will require more electrical stimulation. The insensitivity of MrgprA3$^+$ neuron to electrical stimulation may be a way in which itch neurons transmit itch information.

Modulation factors of MrgprA3

Chronic pain and pruritus can modulate the expression and function of MrgprA3$^+$ neurons in DRG. Excitability of MrgprA3$^+$ neuron enhanced significantly in chronic inflammatory allergic contact dermatitis model mice which were treated by squaric acid dibutylester. The results showed that it display more depolarized the resting membrane potential, increased the number of action potentials, decreased rheobase, and greater number of action potentials at twice rheobase in MrgprA3$^+$ [26]. In the neuropathic pain model, 26.7% of the MrgprA3$^+$ neuron display spontaneous firing by increasing in the excitability of MrgprA3$^+$ neuron [27]. Furthermore, Chemokine CXCL10 is a molecular that closely involved in itch. MrgprA3$^+$ neurons in contact dermatitis model mice are more sensitive to chemokines CXCL10 compared with normal mice and the proportion of cell increase which response to Chemokine CXCL10 [28]. Moreover, the expression of MrgprA3 was increased by activation of BRAF which is a serine / threonine kinase that activates ERK by the RAF / MEK / ERK pathways [29]. However, Psoriasis model mice have a strong scratching behavior, the expression of MrgprA3 in psoriasis mice does not increase [30].

MrgprA3 in itch

MrgprA3 as a specific itchy marker

Is there a subset of neurons that transmit itch from peripheral fiber to central nerve system? This is the problem that promotes researchers to think about and focus on issues. The research group from Dong Xinzhong in HHMI showed that MrgprA3$^+$ neurons are a subpopulation of chloroquine-responding neurons and induced the scratching behavior by chloroquine. MrgprA3 has been identified as an itch receptor [9]. Using Cre- technique cross MrgprA3-Cre line with several Rosa26 reporter lines, then unambiguous expression of red fluorescent protein tdTomato in MrgprA3-positive neurons (Mrgpra3GFP-Cre; ROSA26td Tomato mouse). These MrgprA3-positive neurons can be found by red fluorescent. The electrophysiological recording in vivo, MrgprA3$^+$ fibers responded to stimulation of many pruritogens, such as histamine, capsaicin, bovine adrenal medulla 8-22 peptide (BAM8-22), chloroquine and cowhage spicules. However, another pruritogen, MrgprD agonist β-alanine, does not evoke the responses of MrgprA3$^+$ fiber [9]. When the MrgprA3$^+$ neurons were ablated, the scratching of mice in acute or chronic itch model significantly reduced, but no effect on pain behavior. The above data suggests that MrgprA3$^+$ neurons mediated a special itchy sensation.

TRPV1 is a nociceptive receptor which involve in pain behavior. Is this molecule related to MrgprA3? Activation of TRPV1 channels

on MrgpA3 positive neurons causes itching or pain? Han et al. used immunohistochemistry to confirm that TRPV1 and MrgprA3 were expressed on the same neurons. Meanwhile, a Cre- genetic technique also was adopted to selectively express TRPV1 on MrgprA3^{+} positive neurons and ablate TRPV1 on MrgprA3^{-} positive neurons [9]. They found that MrgprA3^{+} neurons response to both the pruritogen chloroquine and the normally painful stimulator capsaicin by imaging and electrophysiological experiments. Furthermore, interestingly, injection of chloroquine and capsaicin cause itch behavior rather than pain behavior. So, MrgprA3^{+} neurons are a class of specific itch neurons

MrgprA3 mediates pruritus induced by chloroquine

Chloroquine is a common ant malarial drug, but also the treatment of rheumatoid, lupus erythematosus and other diseases. One of chloroquine side effects is pruritus. Interestingly, some malaria patients in Africa have even refused to take chloroquine to treat malaria because the itch induced by chloroquine is more unbearable than torment of malaria. Therefore, this caused researchers pay more attention to chloroquine-induced itch [31,32]. The researchers found that chloroquine-induced itch was not allergic symptoms, and antihistamines drug was almostly ineffective. Then the researchers move on look for histamine independent receptors activated by chloroquine. Injection of chloroquine in the normal mice and Mrgpr-cluster $\Delta^{-/-}$ mice, the behavior test results showed that the total number of scratching bouts significantly less in Mrgpr-cluster $\Delta^{-/-}$ mice than normal mice. However, Injection of histamine and 48/80 to the neck of Mrgpr-cluster $\Delta^{-/-}$ mice did not decrease the number of scratching compare with wide type mice.

The results indicated that Mrgprs play a role in chloroquine-induced itch. Subsequently, the researchers used calcium imaging and electrophysiological methods to examine the responses of DRG neurons to chloroquine. About 5% of DRG neurons from wide type mice respond to chloroquine, but the neurons of Mrgpr-cluster $\Delta^{-/-}$ mice do not completely respond to chloroquine [7]. Furthermore human embryonic kidney (HEK) 293 cells were individually transected into 12 Mrgprs that were deleted in Mrgpr-cluster $\Delta^{-/-}$ mice. It was confirmed that MrgprA3, MrgprA1 and MrgprX1 among 12 Mrgprs responded to chloroquine, and only MrgprA3 exhibited a strong response to chloroquine [7]. Responsiveness of MrgprA3 to chloroquine was eliminated by MrgprA3 against siRNA application [7].Together; these data strongly showed that MrgprA3 and MrgprX1 are chloroquine-induced itch G protein-coupled receptors.

The ionic mechanism and signal pathways of MrgprA3 in itch

TRPA1 is an ionic channel couple of MrgprA3. When TRPA1 was activated, it opened and induced sodium and calcium influx into the cell. In 2011, Willson used calcium imaging and biochemical methods to find out that a subpopulation of neurons selectively expressed MrgprA3, TRPA1 and TRPV1, which response to stimulator chloroquine [12]. Moreover, cultured neurons isolated from TRPV1$^{-/-}$deficient mice exhibited no difference in the magnitude of the Ca^{2+} influx which response to chloroquine. But chloroquine induced response is inhibited in cultured neurons isolated from TRPA1$^{-/-}$ deficient mice. It is confirmed that TRPA1 mediated activation of chloroquine coupled to MrgprA3 through the cell line. In behavior test, and chloroquine induced srcatching behavior decreased significantly in TRPA1$^{-/-}$deficient mice [12]. Studies suggest that chloroquine activated MrgprA3 through the intracellular signaling pathway coupled to TRPA1, opened the channel and cause calcium influx, depolarized cell to threshold to produce action potential. GPCR signaling leads to the dissociation of Gα and Gβγ which has been demonstrated to modulate ionic channels. Applying galleon to DRG neurons which are a small molecule inhibitor of Gβγ, markedly decreased both the magnitude of chloroquine-evoked Ca^{2+} signals and action potential firing [12]. These findings suggest that Gβγ signaling is required for the process of Mrgpr A3 coupling to TRPA1.

It has also been shown that Gβγ signaling open channels by PLC. But the PLC inhibitor U73122 does not change the magnitude of chloroquine-evoked Ca^{2+} signals. In addition, scratching bouts induced by chloroquine did not decrease in the mice which was knocked out the PLCβ [33]. finally, these data show that PLC does not participate in CQ activated MrgprA3 itchy signal transduction. TRPC3 is anther TRP channel mediated chloroquine activated MrgprA3 response in other subpopulation neurons which only express MrgprA3, but not TRPA1. TRPC blockers BTP2 and TRPC3 blocker Pyr3 can inhibit chloroquine induced responses [24]. What is more, MrgprA3 enhanced the sensitivity of TRPV1 in the sensory neuron. MrgprA3 and TRPV1 are co expressed. Preliminarily perfused with chloroquine enhance the magnitude of capsaicin -evoked Ca^{2+} signals. This increasing of sensitivity is linked to the PLC-PKC signaling pathway. PLC blocker U73122 and PKC blocker BIM had an effect on the hypersensitivity by activation of MrgprA3. In contrastly, activation of MrgprA3 reduced the sensitivity of TRPM8, which may be activation of Gq [24]. Another study found that after activation of Mrgpr A3 by chloroquine, TRPA1 channels opened, and QX-314 (a membrane-impermeant sodium channel blocker) entrance into neurons with Ca^{2+} signals which suppress sodium channel currents. Scratching behaviors were significantly reduced by applying chloroquine again [34]. Therefore, after sufficient activation of TRPA1, administration of QX-314 may be a new strategy for effectively treating pruritus.

MrgprC11

Expression and distribution of MorgprC11

Since cluster is characteristic of Mrgprs gene, MrgprC and MrgprA gene subgroups are clustered on the same chromosome. Distribution of MrgprC11 gene is same as MrgprA3 on the chromosome 7[15]. Zylka reported that MrgprC11 is expressed only on small diameter neurons of DRG and TG, and it do not express in the central nervous system and other organs [22]. In accounting for about 3% of all DRG neurons are MrgprC11 positive neurons [35]. MrgprC11 is not a member of humanity in which there are only 10 Mrgprs. However, hMrgpr X1 sequence is highly homologous with Mrgpr A3 and Mrgpr C11 of mice, which is similar to 54% of Mrgpr C11 [36].

Property of MrgprC11^{+} neurons

MrgprC11^{+} neurons are mostly IB4^{+} non-peptide neurons in mice and rat [22]. 46% of MrgprC11 is co-expressed with IB4 [37]. MrgprC can also labeled peptide markers in DRG, only a small proportion, 11% of MrgprC11^{+} neurons co-express for CGRP, 10% of MrgprC11^{+} neurons co-express for SP. Further, MrgprC11^{+} neurons of mice do not express purinergic receptor P2X3. However, the expression of MrgprC11^{+} neurons in rat is different from mice, of which MrgprC11^{+} neurons expresses P2X3 [22]. In addition, most of all MrgprC11^{+} neurons express MrgprA3, suggesting that MrgprC11 play a role in itch. Similarly to MrgprA3^{+} neurons, MrgprC11^{+} neurons do not have much overlap with MrgprD [9,22]. Interestingly, only a few mouse MrgprC11^{+} neurons have expression of TRPV1. TRP channels possibly such as TRPA1 with MrgprC11 form GPCRs-ion channel axis, but another report suggests that approximately 52% of TRPV1 and MrgprC co-expressed in rats [37].

MrgprC11 in itch

MrgprC11 was activated by BAM8-22 induced itch

Researchers interesting in the role of MrgprC11 in itch for high proportion co-expression of MrgprC11 and MrgprA3. Bovine adrenal medulla 8-22 peptide (BAM8-22) is a proteolytically cleaved production of proenkephalin A, which is an MrgprC11 agonist. Liu demonstrated the BAM8-22 can induce pruritus by activated MrgprC11in mice. 50 μl 50 nmol/L of BAM 8-22 was injected subcutaneously into the neck of Mrgpr-cluster $\Delta^{-/-}$ mice and wide type mice, 80 scratching bouts were observed in wide type mice , while the scratching behavior of Mrgpr-cluster $\Delta^{-/-}$ mice was significantly decreased [7]. In calcium imaging experiments, approximately 3.6% of DRG neurons in wide type mice responded to BAM 8-22, however, BAM 8-22 cannot induce change of calcium signal in the neurons of Mrgpr-cluster $\Delta^{-/-}$ mice [7]. What is more, transfection of exclusively MrgprC11 or hMrgpr X1 in HEK293 cells, these cells responded to stimulation of BAM 8-22 [7].

BAM 8-22 can not only induce scratching in mouse, but also can evoke the sensation of itch in human. Sikand directly observed the volunteer response to BAM 8-22, all volunteers feel itch and desire to scratch when fore arm epidermis were stabbed by the bean which was soaked in BAM8-22 solution, but histamine receptor antagonist cannot block this response [38]. It is suggesting that BAM8-22 activation of hMrgprX1-induced itch which is non histamine dependent itch. The above study showed that hMrgprX1 and MrgprC11 mediate BAM8-22-induced itch. Furthermore, it has been reported that BAM8-22 play a part of analgesic effects by activated MrgprC11 [39]. Presynaptic N-type calcium channels were inhibited by activation of MrgprC11in DRG neurons that did not release the pain neurotransmitters and transmitted pain signal [40,41].

MrgprC11 mediates SLIGAL-induced itching

Ser-Leu-Ile-Gly-Arg-Leu (SLIGRL) is a small synthetic peptide. It has been reported that SLIGRL can induce scratch in mouse by activation protease activated receptors 2 (PAR2) in previous studies [42]. In chronic itch model, mice responded to SLIGRL sensitively and sensitization to SLIGRL can be blocked by PAR2 receptor blockers [43]. However, inconsistently with previous studies, scratching bouts are no difference induced by projected SLIGRL to normal mice and *PAR2*$^{-/-}$ mice. This data suggests that SLIGRL may not evoke scratching by directly activating PAR2. Further studies showed that SLIGRL induced scratching behavior significantly decreased in Mrgpr-cluster $\Delta^{-/-}$ mice than normal mice [35].What is more, the genes which were knocked out in Mrgpr-cluster $\Delta^{-/-}$ mice were transferred into CHO cells one by one. Only cells expressing MrgprC11 responded to SLIGRL, indicating that SLIGRL induced by activating MrgprC11 [35].

MrgprC11 mediates cathepsin S-induced itching

Cathepsin S is a member of the family of papain which is not only a cysteine protease, but also a lysosomal enzyme. The lerner research group reported in 2010 that cathepsin S activates induce itch by activated PAR2 [44]. In addition, Cathepsin S is also a marker of some related itching-related diseases, such as atopic dermatitis [45]. Further studies have shown that MrgprC11 is involved in itch induced by Cathepsin S. MrgprC11can selectively activated by Cathepsin S on the or hela cells, but fewer DRG neuron from Mrgpr-cluster $\Delta^{-/-}$ mice response to Cathepsin S [46]. Administration of Cathepsin S evoked scratching in Mrgpr-cluster $\Delta^{-/-}$ mice was significantly less than wide type mice, surprisingly Cathepsin S also evoked scratching in the knockout of the PAR2$^{-/-}$ mice. This suggests that MrgprC11 directly mediates Cathepsin S-induced itching behavior. Cathepsin S evoked the cleavage of the MrgprC11 N-terminus. Reddy synthesizes a series of peptides according to the cleavage point near N-terminal, but these peptides failed to induce calcium responses in MrgprC11-expressing cells do not activate MrgprC11 that is not consistent with other PAR2 N-terminal peptides [46]. It is suggested that Cathepsin S directly changed the MrgprC11 extracellular domain or changed in internal structure leading to evoke signal and delivery by cleaving the N-terminal portion of MrgprC11. In addition, substitution with N-terminal of other GPCR to the MrgprC11 extracellular cleavage site, mutant MrgprC11 still response to Cathepsin S which cannot directly activate other receptor fragments. In a word, the cleavage site is more important than tethered or diffusible ligand in Cathepsin S induced response.

The mechanism and signal pathways of MrgprC11 involved in itching

TRPA1 is the downstream ion channel of MrgprC11 on the neuron which co-expressed TRPA1, TRPV1 and MrgprC11. Fewer DRG neuron response BAM8-22 from TRPA1$^{-/-}$ mice or pre-perfused TRPA1 blocker HC030031 from wide type mice. $G_{q/11}$, not $G_{i/o}$ or Gs coupled to activation of Mrgpr C11 calcium-signaling pathway [36]. In addition, Gβγ blocker gallein does not block the effect by BAM8-22-activated Mrgpr C11. It is suggesting that Gβγ does not participate in the signaling pathway of MrgprC11-TRPA1 [9]. Furthermore, BAM8-22 evoked response by activation of Mrgpr C11 was blocked by PLC blocker U73122. Interestingly, TRPV1 may also be present in the MrgprC11 signaling pathway. MrgprC11 and TRPV1 co-transfected cells response to BAM but amplitude

was lower than MrgprC11 and TRPA1 co-transfected cells [9]. On the contrary, activation of MrgprX1 with BAM8-22 enhances the sensitivity of TRPV1.

Pre-perfusion of BAM8-22, TRPV1 response increased to capsaicin or H^+ ions, and elevated temperatures [47]. The calcium channel is the downstream channel couple to MrgprC11 yet. The agonist JU58 activates MrgprC11 to inhibit high-voltage activated calcium channel (HVA), which is a N-type calcium channel rather than L- or P/Q- type. PLC participates in activation of MrgprC11 coupled to N-type calcium channel. Furthermore, Gαi and Gαs blockers cannot block the response by activation of MrgprC11, it is indicated that HVA currents were inhibited by activation of Mrgpr C11 that is independent of Gαi and Gαs [40,41]. Although the cell pathway mechanism of MrgprC11 has been studied, there is still a class of cells on which MrgprC11 does not co-expressed with TRPA1 and TRPV1. Activation mechanism of Mrgpr C11 is not clear in this type of cell and needs further study.

MrgprD

MrgprD gene, protein distribution and expression

In human, the MrgprD gene was located on chromosome 11. The Location of rat and mouse MrgprD is chromosome [15,18,22]. Although property of rat or mouse Mrgprs gene is clustered, MrgprD does not cluster with MrgprA, MrgprC and other subgroups, but with MrgprE, F, G clusters [22].MrgprD protein expression is similar to MrgprC11 and MrgprA3, which exclusively expressed on small diameter neurons of DRG and TG [15,22]. $MrgprD^+$ nerve fibers are no peptide, unmyelinated, mechanically sensitive C fibers, which project to the skin and spinal cord. In the peripheral skin, $MrgprD^+$ fibers only innervate the hair and hairless skin, but muscle, and blood vessels without the distribution of $MrgprD^+$ fibers [48]. Other organs such as the cornea, meanings, stomach, intestine, heart, bladder, etc. do not express $MrgprD^+$ fiber [48]. $MrgprD^+$ fibers are very regular in the projection of the skin, only projecting to the skin granule layer.

In addition, distribution of $MrgprD^+$ fibers are strictly different with $CGRP^+$ fibers which projected to the stratum spinosum [9,48]. Fewer $MrgprD^+$ fibers are intertwined with $CGRP^+$ fibers. It is suggesting that there is a difference between nonpeptide fibers and peptide fibers in the spatial distribution and function. At the spinal cord, the spatial distribution of MrgprD+ fibers is similar to periphery. MrgprD+ fibers projected to the spinal dorsal horn cellular lamina IIo and IIi, which was named II middle. lamina II middle does not overlap with the I-layer and IIo layers where by $CGRP^+$ fibers projected, but overlap with the IIo and IIi layers was projected by $IB4^+$ fibers [48].

The relationship between $MrgprD^+$ neurons and other receptors and channels

Mostly of rats and mice, $MrgprD^+$ neurons were no peptide neurons, 52% $c\text{-}RET^+$ neurons and 64% $IB4^+$ neurons expressed MrgprD [48]. In addition, $MrgprD^+$ neurons did not express peptide marker CGRP [22]. but, about 60% of $P2X3^+$ neurons express MrgprD. Furthermore, for MrgprD unco-expressed with MrgprC, MrgprA subgroup, $MrgprD^+$ neurons and $MrgprsA3^+$ did not overlap [9,22]. The expression of TRPV1 in $MrgprD^+$ neurons was similar in different rodents. TRPV1 was not expressed in the $MrgprD^+$ neurons of rats but not in mice.

MrgprD is involved in itching

MrgprD mediates β-alanine-induced itching

β-alanine is a natural amino acid, can promote muscle growth and recovery, but oral or injection of β-alanine can induce scratching behavior. In 2012, Liu et al. showed that MrgprD mediates β-alanine-evoked itch [8]. Oral consumption and subcutaneous injected β-alanine can induce cause a dose-dependent scratching behavior. However, injection site of human skin also no flare or wheal, suggesting that β-alanine induced itch is histamine independent itch. As early as 2004, Shinohara found that β-alanine was an [49]. The number of β-alanine induce scratching behavior decreased in $MrgprD^{-/-}$ mice. Furthermore, calcium and electrophysiology study showed that β-alanine could directly activate $MrgprsD^+$ DRG neurons. About 12% of DRG neurons in wide type mice response to β -alanine, however, β-alanine-activated neurons cannot be activated by chloroquine and histamine. What is more, $MrgprD^{-/-}$ mice neurons have no response to β-alanine [8]. In addition, studies have reported that the excitability of $MrgprsD^+$ neurons increases in the atopic dermatitis mice which may be one of the factors causing scratching of atopic dermatitis [26].

MrgprD involved in itch's ion channel mechanism and signal pathway

The potassium channel is a novel coupling channel of MrgprD. Crozier reported that activation of Mrgpr D could inhibit low threshold, slowly activating, non-inactivating potassium channels [50]. This potassium channel comprises of the KCNQ2/3 subunit, which was activated and induced in large part to M-current. β-alanine inhibited M current from CHO cell on which KCNQ2/3 with MrgprD were co-expressed and excitability of MrgprD positive cell increased. Furthermore, β-alanine induced response was blocked by PLC blocker U73122 and edelfosine, suggesting that PLC was involved in the signal pathway of MrgprD. In addition, pertussis toxin can block the response caused by β-alanine yet, demonstrating that Gq and Gi are coupled with Mrgpr D. what is more, Ca^{2+} activated chloride channel is a channel for coupling of Mrgpr D too. MrgprD expressed on xenopus oocytes response to β-alanine, which can be blocked by Ca^{2+} activated chloride channel blocker FFA [51]. Additionally, PLC inhibitors or IP3 receptor antisense oligonucleotides can inhibit the inward currents evoked by β-alanine, nevertheless, the DAG-PKC pathway blockers cannot block this response, suggesting that β-alanine activates Ca^{2+} activated chloride channel by the Gq-PLC-IP3 pathway.

Other Mrgprs in itch

In addition to MrgprA3, MrgprC11, MrgprD involved in itch, MrgprB2, MrgprA1 may play a role in pruritus. In the connective tissue, mast cells are activated to release histamine, various inflammatory and immunomodulatory substances and other pruritogen. McNeil reports that the mouse MrgprB2, and human

MrgprX2 specifically expressed on the mast cells in skin, intestine, trachea [52]. In addition to IgE antibodies can activate mast cell to release histamine. And drugs or other secretagogues can activate MrgprB2 / MrgprX2 of mast cell and induced histamine release. Red neck syndrome, also known as red man syndrome, is a drug-induced side effect. It is shown that when rapidly intravenous administration of drugs, face and neck was red and accompanied by a strong scratching [53]. Generally, it was considered that drug or drug metabolites induced histamine release from mast cell. Many drugs can induce red man syndrome, such as vancomycin, teicoplanin, and rifampicin and so on. Interestingly, vancomycin induced red man syndrome by activated MrgprX2 on the mast cell. QWF, an MrgprX2, NK-1, MrgprA1receptor blocker, can block vancomycin's response [53].

Substance P is a small peptide that induced scratching by activated the post synaptic neurokinin 1 receptor which is a classic receptor for substance P in an earlier study [54]. However, Azimi recently have reported that substance P -induced itch does not just link to NK-1 receptors, but also activated a member of Mrgprs family -MrgprA1 on the DRG neurons [55]. Subcutaneous injection of substance P, Mrgpr-cluster $\Delta^{-/-}$ mice significantly decreased scratching. Further, scratching behavior induced by injected substance P to the neck of wide type mice is more compared with the Mrgpr-cluster $\Delta^{-/-}$ mice. MrgprA1 receptor blockers can block substance P-induced itch, but blocking effect of NK-1 receptor inhibitor is not efficacious. In order to exclude the effect of MrgprA1 on the substance P-induced itch, applying substance P to the cultured DRG neurons from *NK-1R*$^{-/-}$ mice, the cultured DRG neurons still response to substance P induced calcium flux which was blocked by the MrgprA1 receptor blockers QWF. In conclusion, MrgprA1 receptor was involved in substance P-induced itch.

Future Prospects

Itch or pruritus is not only a disease but also a clinical symptom of many diseases. Mechanism of Itch is unclear and complex, a lot of endogenous molecules and exogenous substances can induce scratching behavior. A variety of receptors, ion channels, and cytokines are involved in mediating itch. Looking for itching - related receptors, ion channels, elucidating their cellular and molecular mechanism and understanding their function in itch has been a highlight for itch research. For instance, Mrgprs receptors are candidate members among those pruritic receptors. Activation of MrgprA3 mediated histamine independent chloroquine-induced itch, but MrgprA3^{+} neurons also respond to histamine which express histamine receptor, suggesting that the cellular mechanism of itch is complexity, and theory classification of itch may be improved which was divided into histamine and histamine independent.

Furthermore, although many MrgprC11 itch sensory ligands were discovered, distribution characteristics, projection and electrophysiological characteristics of MrgprC11^{+} neuron remain to be further studied. Moreover, the downstream channel of MrgprD^{+} neurons is also indistinguishable. What is more, expression of MrgprB2 in mast cells shows that Mrgprs may be a bridge between the nervous system and the immune system, which are involved in the pruritic induction of scratching. In addition, a variety of other Mrgprs receptor exclusively distributed in the peripheral nervous system, whether they are involved in itchy function? Can those endogenous or exogenous pruritic molecules activate these receptors? In a word, these may be the next of itch studying. The further studies of the Mrgprs receptors will lay a solid foundation for us to promote understanding itching mechanism and develop new antipruritic drugs.

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a. Gβγ and TRPA1 are required for MrgprA3 mediated pruritus induced by chloroquine

b. TRPC3 is anther TRP channel mediated chloroquine activated MrgprA3 response.

c. BAM8-22, SLIGRL and cathepsin S activated MrgprC11 leading to activate PLC and TRPA1 via the Gq G-protein.

d. The MrgprD is coupled to stimulation of PLC by the Gq and Gi protein leading to production of the second messenger IP3. In DRG neurons, activation of MrgprD can lead to inhibit slowly activating, non-inactivating potassium channel and open Ca2+ activated chloride channel.

e. MrgprA1 receptor was involved in substance P induced itch. However, which channel coupled to MrgprA1 is unclear.

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