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Kinesins: Motor Proteins as Novel Target for the Treatment of Chronic Pain

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Abstract
Kinesins are one of the neoteric and efficacious targets recently reported to play an important role in the initiation and progression of chronic pain. Kinesins are anterograde microtubule-based motor proteins that are involved in trafficking of receptors including nociceptors and progression of pain. The specific kinesin and regulatory proteins interplay is crucial for the delivery of nociceptors to the synapse. If this complex and less understood interplay is inhibited, it may result in a decrease in central sensitization, and thus attenuation of pain. This review is focused on the transportation process of receptors/cargos, the role of regulatory proteins influencing the respective kinesin, and their relationship with chronic pain. The review also features specific strategies adopted by researchers for targeting kinesin and chronic pain. Considering the recent preclinical success of kinesin inhibition in pain, it is expected that inhibitors for kinesin or enzymes responsible for kinesin activation could be developed or repurposed as alternative, safe, and potential therapies for the treatment of chronic pain.

Keywords Bone cancer pain · Calcium calmodulin kinase 2 · Cyclin-dependent kinesins · KIF13B · KIF17 · NMDA, pain · Sodium channels · TRPV1

Introduction

Chronic Pain: a Perpetual Problem with Limited Therapies

Even after more than a century of studying pain and its underlying mechanisms, we are unable to develop the effective therapies against it. Recent studies indicate that around 20–25% of world's population suffer from some kind of chronic pain out of which 35% do not respond to currently available drugs in clinics [1]. Chronic pain is a highly prevalent disease with astounding health care cost globally. It has become the leading cause of disability in the USA and the seventh leading one worldwide [2]. Moreover, the therapies which are available either leads to inadequate pain relief or causes severe side effects including but not limuted to drug addiction, hallucinations, respiratory depression, constipation, and even death in some cases [3].

Pain, as International Association for the Study of Pain (IASP) defines, is an unpleasant sensory and emotional experience which is usually associated with actual or potential tissue damage. Or, in other words, pain is a protective mechanism which acts as an alarm and warns us against potent noxious stimuli. Pain induces behavioral changes such as fear, anxiety, avoidance anticipation, and learning [4]. Pain perception works through specialized noxious receptors, which are localized at nerve endings of primary nerve fibers like Aδ and C fibers [5, 6]. These specialized noxious receptors, such as transient receptor potential (TRP) family of receptors, acid-sensing ion channel (ASI), sodium channels, and various purinoceptors, etc., sense the wide range of chemical, mechanical, as well as thermal stimuli. These get activated and depolarize the primary afferent neurons and the signals are passed on to higher centers of spinal cord and brain [7, 8]. This is

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physiological pain and has a protective role by minimizing the damage through inducing behavioral changes [9].

Nociceptors sometimes get activated non-specifically through exposure to various bacterial toxins and inflammatory mediators, as a result that results in lowering the threshold of primary neurons due to their repeated activation and upregulation, causing peripheral sensitization [10]. These sensitized primary neurons send incorrect signals to neurons in the spinal cord and brain where a similar reduction in the threshold is observed due to repeated activation and upregulation of excitatory receptors. This phenomenon is called central sensitization [11, 12]. Both peripheral and central sensitizations lead to the slow, persistent development of thermal hyperalgesia (increased sensitivity to heat) and mechanical allodynia (increased sensitivity to touch) [12]. The mechanisms behind receptor dysregulation during chronic pain is not fully explored yet. Recent findings from several groups indicate the involvement of kinesins in upregulation of nociceptors. In this review, we will discuss the several recent findings indicating potential role of different kinesins in the progression of chronic pain.

Kinesins: a Freight Train for Cargos

Kinesins are microtubule-based motor proteins that are generally involved in the anterograde transport of the cargo. Motor proteins, as they move along the substrate, convert chemical energy into mechanical energy by the hydrolysis of ATP [13]. These motor proteins move along the neuronal highways called "microtubule" and alleys called "actin." Based on the pathway motor protein used, these motor proteins are classified into two classes—(1) motor protein which travels through actin-myosin (actin-based motility) and (2) motor proteins that travel through microtubule, i.e., kinesins and dyneins (tubulin-based motility). Most types of kinesin, such as kinesin N-terminal (amino acid-terminal) and M-terminal (motor-terminal) generally move towards the positive end (the rapidly growing end) of the microtubule, except for the C-kinesin (carboxy-terminal) family motor proteins [14]. For example, non-claret disjunctional (NCD) is a negative-end-directed motor protein C-kinesin that is involved in chromosomal segregation during cell division [15]. Dyneins are another class of tubulin-based motor proteins, which always move towards the negative end (slow-growing end) of the microtubule [16].

A cell that may be a neuron in origin has a cytoskeleton that is composed of intermediate filaments, actin/myosin filaments, and microtubules. These three major protein fiber systems form cytoskeleton, contribute to structure maintenance and various functions [17, 18]. These microtubules and actin serve as tracks for the passage of cargo. Microtubule is used for transport of cargos across long distances from the nucleus to neuronal membrane present at synapse, whereas actin is used for the movement of cargo across small distances [13].

Cargos, in the form of large protein complexes or receptor, are synthesized by the nucleus and rough endoplasmic reticulum packed into vesicles by Golgi body. These synthesized protein complexes/receptors get diffused into cytoplasm, bind with kinesins leading to activation of kinesin [19]. Kinesins, present in the cytoplasm in the inactive form, are activated in the presence of cargo. In the absence of cargo, these kinesins are kept in an inactivated state so that there is no dissipation of ATP. This inactivated state is the result of a phenomenon called autoinhibition [20]. In inactive state, kinesins exist in a folded conformation. This autoinhibition is achieved by two mechanisms, first by binding tail domain to motor domain and the second by inhibition of progressive motility, due to direct binding of coiled-coil (CC) segment with the motor domain. After activation, kinesins bind to microtubule and process of transportation of cargo starts. These cargo are found in association with other motor proteins such as dyneins and myosins [21, 22].

The kinesins move along the microtubule, whereas myosin moves along actin. Since microtubule branch into various actin branches, myosin, which is attached to cargo, tends to cling to these actin sidetracks and this results in a tug of war the dominant force prevails. Similarly, the tug of war also results in dyneins, which move in opposite direction negative end of the microtubule, same as before the dominant force prevails and cargo moves in that direction [23]. When kinesins reach near the synapse, regulating proteins play an important role in the delivery of cargo to the synapse. A synapse is a space between the two neurons where two neurons interact with each other. Receivers in the form of receptors are present at the other end of the synapse. The kinesins-cargo complex interacts with these regulating proteins, and when needed at the synapse, cargo is handed over to myosin V [24, 25]. Myosin V then moves along actin to transfer cargo to the synapse. In the case when receptors are transported, disassociation of motor-cargo complex takes place leading to surface expression of receptors such as NMDA during chronic pain [26].

Role of Regulatory Proteins

These are the proteins that are involved in the regulation of transport of goods across the cell. It involves initial events which include (1) motor-cargo complex formation, (2) activation of motor protein and selection of microtubule track, and (3) final stage regulatory events that include the release of cargo on reaching the destination and the recycling of motors [27]. The Rab family and GTPases positively regulate motor-cargo binding.

On the other hand, the MAPK (mitogen-activated protein kinases) family negatively regulates axonal transport and intraflagellar transport. It has been proven that stimulation of the MAPKKK or MAPKK enzymes results

in dissociation of the Kinesin-1-JIP-cargo protein complex [28]. Other pathways, like the Jun N-terminal kinase (JnK), along with the MAPK signaling pathway, are involved in regulation of kinesin-1 [29]. For example, in disease state/neuronal injury, it causes a shift from kinesin-dependent–anterograde transport to dynein-dependent–retrograde transport. JnK signaling is suggested to be involved in the release of kinesin-1 from the vesicles [30].

Microtubule Track Selection

Once the motor protein complex is formed and kinesin is activated, the next step is a selection of track, which depends upon the state of the microtubule [31, 32]. For example, kinesin-1 depends upon the post-translation modification of tubulin subunits. Kinesin-1 generally shows a preference for an acetylated form of α-tubulin, whereas detyrosinated α-tubulin gives the direction kinesin-1 to a specific destination inside the cell [33, 34]. Kinesins have highly conserved motor domains. They react differently to different post-translation modification and heterogeneity of microtubule. Kinesin-3 and Kinesin-2 families have the least affinity for acetylated or detyrosinated microtubules in fibroblasts [31]. Kinesin-3 movements are influenced by polyglutamylation of tubulin subunits [35].

Kinesin Family

There are 45 kinesin genes involved in the coding of kinesin motor proteins. These may generate twice as many of kinesin isoforms, depending on different treatment of synthesized mRNA generated from these genes (difference in splicing will create different kinesin from the same gene sequences). This explains why kinesins are different, but they perform similar kind of function. The generations of isoforms of kinesins are the result of the difference in the pretreatment of mRNA [14] (Tables 1 and 2).

Different Kinesins and Their Role in Pain Regulation

Role of KIF17 in Pain: Ca^{2+}/Calmodulin-Dependent Protein Kinase II Induced Modulation of NMDA Receptors

For a long time, NMDA receptors have been known to be involved in the learning and memory. The upregulation of synaptic NR2B directly affects the induction of long-term potentiation (LTP) and sustained synaptic plasticity. This process is responsible for learning and memory. The increase in KIF17 parallels the upregulation of synaptic NR2B. These two biological processes may have an effect on the learning and memory [46] (Fig. 1). In bone cancer pain, there is upregulation of NMDA receptors in the spinal dorsal root ganglion (DRG) [26]. NMDA is the calcium-permeable ion channel that causes an increase in Ca^{2+} ion concentration. In turn, it activates calcium/calmodulin-dependent protein kinase II (CaMKII) and results in activation of cAMP response element binding protein (CREB). The activation of CREB along with Nuclear Respiratory Factor-1 (NRF-1) and other transcription regulators results in the increased transcription of NR2B and KIF-17. This results in increased NR2B and KIF-17 proteins in the cytoplasm. Evidences suggested key role of CaMKII in nociceptive transmission. CaMKII preferentially express in the pain processing regions such as lamina II of dorsal horn spinal cord and dorsal root ganglion [47] CaMKII upregulated in spinal cord and DRG after nerve injury and inflammation in the peripheral tissues [48]. CaMKII kinase also phosphorylates NR2B and KIF-17, and this results in the formation of a motor protein complex with mLin-10 as a linker protein. After the formation of the motor-protein complex, NR2B receptors are transported to synaptic membrane, thus increasing the surface expression of NR2B receptors. This increased surface expression results in mechanical allodynia and thermal hyperalgesia. This pathway is not only expressed in cases of bone cancer pain, but also most likely in cases of neuropathic pain as well.

A study by Liu et al. [43] proved that CaMKII attenuates bone cancer pain by inhibiting transport of KIF17/NR2B. The study was done in a murine model of bone cancer pain by implanting NCTC 2472 cells into intramedullary space in the femur of C3H/Hej (20–30 g) mice. KN93, a CaMKII inhibitor, was administered intrathecally in rodents 14 days after tumor cell implantation (TCI) surgery. It was observed that inhibition of CaMKII resulted in a decreased expression of KIF17 and NR2B receptors in a dose-dependent manner and the decreased expression of NR2B receptors was associated with attenuation of bone cancer pain.

Role of KIF13B in Pain: Translocation of TRPV1 Receptors

TRPV1 receptors are highly expressed in peripheral neurons involved in thermal hyperalgesia. These are non-selective Ca^{2+} channels. The presences of functional TRPV1 receptors are essential for heat hypersensitivity [49, 50]. PKC-stimulated SNARE-dependent exocytosis and Src-dependent TRPV1 phosphorylaltion are involved in inflammation-induced rapid membrane insertion of TRPV1 onto synaptic membrane from the vesicular pool [51–53]. Kinesin-13B (KIF13B) is involved in the transport of TRPV1 to the synaptic membrane of the peripheral neurons from Golgi apparatus. Cyclin-dependent protein kinase-5 (CDK-5), an important protein kinase, along with a p35 important promoter of CDK-5 are involved in the

Table 1 Classification of kinesins and their physiological role

Family	Family members	Nature of kinesin	Functions	Regulatory enzyme/proteins involved	Remarks/kinesin-associated with pain	Animal model and drug/ molecule used in preclinical study	References
Kinesin-1	KIF-5A KIF-5B KIF-5C	Homotetramers consisting of kinesin light (KLC) and kinesin heavy chain (KHC)	Vesicle, organelle and mRNA transport, It is involved in the transport of glutamate, GABA and AMPA receptors	1. Two proteins namely FEZ1 (fasciculation and elongation protein-ζ1) or also called as zygin 1 and JIP1 (Jun N-terminal kinase interacting protein 1) (also called MAPK8IP1). These two proteins bind to inhibitory regions of kinesin present at KHC in main portion and KLC subunits respectively for the activation of kinesins 3. With GRIP1 (Glutamate Receptor Interacting Protein 1) as a scaffold protein, they transport cargos. 3. For the transport of GABA receptors • Huntingtin-Associated Protein 1 (HAP1) (as a linker protein) • Gephyrin and GABA-associated protein (GABARAP) links receptor to microtubule and makes it available for transport • RACK1 (Receptor for activated C-Kinase), GRIF1 (GABAAR-interacting factor-1), PKC-β11, Src, AP2, and AKAP1 (A-Kinase Anchoring Protein 1)	1. Kinesin 5A mutation causes hereditary spastic paraplegias (HSP) 2. Kinesin 5B is involved in transport of Na_v 1.8 channels which is associated with pain	1. Complete Freund's adjuvant (CFA) was injected into hind paws of Sprague-Dawley male rats and maintained for 7 days. 2. shRNA (Short hairpin RNA) that knocks out KIF5B in mice that cause decreased expression of KIF5B	[14, 27, 36–38]
Kinesin-2	KIF3A KIF3B KIF3C	Heterotrimeric subfamily	1. Vesicle, melanosome, intraflagellar transport, transport of receptors 2. KIF-3A and KIF-3B are thought to be involved in left-right axis determination 3. Growth cone elongation 4. KIF3 is involved in the transport of Herpes simplex virus-1 along the axons after reactivating from latency		1. KIF17 is involved in the transport of NR2B receptors which is responsible for mechanical allodynia	Sixty 4–6-week-old adult male C3H/HeJ mice were used. A murine model of bone cancer pain was generated by injecting NCTC 2472 cells into intramedullary space of right femur 5 μl of sense and antisense KIF17 oligonucleotides (ODN) dissolved in saline was injected intrathecally, 5 μl of saline was used as vehicle control. The Antisense KIF17 was able to attenuate the bone cancer pain	[14, 27, 38]
	KIF17	Heterodimers	1. KIF17 is involved in the transport of receptors (e.g., NR2B) responsible for memory	m-Lin10 (linker protein), calcium/calmodulin-dependent protein kinase II			

Table 1 (continued)

Family	Family members	Nature of kinesin	Functions	Regulatory enzyme/proteins involved	Remarks/kinesin-associated with pain	Animal model and drug/molecule used in preclinical study	References
Kinesin-3	KIF1A KIF1B KIF1C KIF13A KIF13B KIF14 KIF16A KIF16B	Dimeric	Vesicle transport and receptor transport, AMPARs (GluA2/3)	CDK-5 enzymes are involved in the association of TRPV1 with kinesin 13B. liprin-	1. Mutation in KIF1B is linked to development in Charcot-Marie-Tooth disease 2. KIF-13B is involved in transport of TRPV1 receptors which are responsible for thermal hyperalgesia	CFA-induced inflammatory model of pain rats used were Sprague-Dawley rats (200–250 g). 5 μl of Roscovitine (100 μg) dissolved in DMSO was administered intrathecally 30 min before administration of CFA. 5 μl of DMSO was administered as vehicle control. The results showed a decrease in the expression of TRPV1 receptors	[14, 27, 20, 38]

phosphorylation of the kinesin-13B (KIF13B) and TRPV1 [44, 45]. Other CDK-dependent phosphorylation pathways may be involved. This causes activation of both KIF13B, which is present in an auto-inhibited state in the cytoplasm, and TRPV1. These activated proteins now form a motor-protein complex. KIF13B, which is now in association with a vesicle containing TRPV1 receptors and possible linker proteins, binds to microtubule and begins to move along microtubule across axon.

A study by Xing et al. [44] proves that cyclin-dependent kinase (CDK-5) are involved in the phosphorylation of KIF13B and result in trafficking of TRPV1 receptors. They used the inflammatory model of pain by injecting 100 μl of Complete Freund's Adjuvant (CFA) into hind paws of Sprague-Dawley rats (200–250 g). The experiments done by the authors proved following points. First, they proved KIF13B is involved in the transit of TRPV1 receptors across the axon by transfecting cultured DRG neurons with Lentivirus (LV) containing shRNA of KIF13B. This knocked down KIF13B in the cultured DRG neurons. Biotinylation assays of TRPV1 receptors indicated that knocking down KIF13B would decrease the surface expression of TRPV1 receptors, indicating KIF13B is required for the transit for TRPV1 receptors. Second, they proved CDK-5 and p35 are involved in phosphorylating KIF13B in Forkhead Associated (FHA) domain of KIF13B. They proved it by co-immunoprecipitating (Co-IP) the GFP-motor FHA, CDK-5 and p35 in CHO, F11 cell line, and also in the cultured neuron of DRG. They performed in vitro cell kinase assay of CDK-5 obtained by immunoprecipitating from rat DRG. The isolated enzyme was incubated with His6 fusion peptide and phosphorylation mix (containing 5 μCi γ-P32 radioactive), the SDA-PAGE sample buffer was added and SDS-PAGE was performed. Cells were then stained, dried, and autoradiographed. Results showed that CDK-5 and p35 phosphorylated KIF13B in FHA domain.

Moreover, authors proved that CDK-5 and p35 phosphorylated KIF13B at threonine 506th position of FHA domain of KIF13B. Authors mutated threonine with alanine and subjected to in vitro cell kinase assay, the mutation abolished the phosphorylation of KIF13B. To prove this in vivo, they constructed KIF13B-TAT fusion peptides. Fusion peptide sequence conjugated with the peptide sequence of HIV-TAT peptide, one control other one was mutated. They administered TAT-fusion peptides (30 μg) 30 min before the administration of CFA into hind paw of rats. Calcium imaging of culture treated with mutated peptide showed delayed calcium uptake. The behavior tests of thermal hyperalgesia indicated an increase in the paw withdrawal threshold of rats treated with the mutated TAT-fusion peptide.

TRPV1 receptors are synthesized by the nucleus and packed into vesicles containing TRPV1 receptors by the Golgi body. After that, vesicle forms an association with

Table 2 Preclinical findings demonstrating different kinesins involved in pain regulation along with molecules targeting the same

Sr no.	Kinesin involved	Target	Receptors transported	Molecules	Animal models	References
1.	Kif17	KIF 17	NR2B	KIF 17 Antisense Oligodeoxynucleotide (ODN)	NCTC 2472 tumor cell implantation (TCI) in mice	[39]
2.	Kif17	Cyclic AMP response element binding protein	NR2B	CREB antisense oligonucleotide (ODN)	CCI surgery	[40]
3.	Kinesin	Microtubule	Non-specific	Colchicine or vinblastine	Sciatic nerve ligation surgery	[41]
4.	Kif17	KIF17 and mLin10 interaction	NR2B	Myr-RC-13	NCTC 2472 cells TCI	[42]
5.	Kif17	Calcium/calmodulin-dependent protein kinase II (CaMKII)	NR2B	KN93 (CaMKII inhibitor).	NCTC 2472 cells TCI	[43]
6.	Kif5A	Mutation in KIF5A inability to associate	N.A	N.A	Hereditary spastic paraplegias (HSP) Charcot-Marie-Tooth disease (CMT) Clinical report	[36]
7.	Kif 5B	KIF 5B	Na_v 1.8	Brefeldin (protein transport inhibitor)	Complete Freund's adjuvant (CFA)	[37]
8.	Kif13B	CDK-5 (est. Thr.506 phosphorylation of kinesin 13B)	TRPV1	Roscovitine (R7772) (CDK-5) inhibitor	Complete Freund's adjuvant (CFA)	[44]
9.	Kif13B	CDK-5 (est. Thr 406 phosphorylation of TRPV1)	TRPV1	Roscovitine (R7772) (CDK-5) inhibitor	Complete Freund's adjuvant (CFA)	[45]

KIF13B by possible linker protein. This KIF13B-TRPV1 complex (motor-cargo association) is facilitated by the phosphorylation of both TRPV1 and KIF13B by CDK-5. KIF13B-TRPV1 complex binds to microtubule and moves along microtubule to transport TRPV1 receptors across the axon. On reaching synapse membrane, this KIF13B-TRPV1 complex dissociates to release the vesicle. The dissociated vesicles are now docked onto the synaptic membrane. This docked vesicle fuses with synaptic membrane thus expressing TRPV1 receptors on the surface of neurons (Fig. 2). Continuous expression of TRPV1 receptors on the synaptic membrane results in thermal hyperalgesia.

The vesicle containing TRPV1 receptors are transported by KIF13B to the synaptic membrane. These are docked onto the synaptic membrane by syntaxin (present on the synaptic membrane), synaptobrevin (present on the vesicle), and Synaptosomal-associated protein 25 (SNAP-25) (present on the synaptic membrane) by binding with the vesicle (Fig. 3). These three proteins syntaxin, synaptobrevin, and SNAP-25, which are involved in docking of vesicles onto the synaptic membrane, form the core complex. Synaptotagmin which is present on the vesicle has two domains C2A and C2B. C2A binds to syntaxin whereas C2B binds to calcium, which causes a disturbance in the phospholipid bilayer and results in the fusion of vesicle to the synaptic membrane. All these proteins involved in docking to the fusion of vesicles are called Soluble N-ethylmaleimide-sensitive factor (NSF) Attachment protein Receptor (SNARE) proteins. The proteins, which are present on the vesicle, are called V-SNAREs (vesicle-SNARE), and proteins that are present on the synaptic membrane are called T-SNAREs (target-SNAREs). This fusion results in the release of contents inside the vesicles (e.g., neurotransmitters) and TRPV1 receptors get expressed onto the surface of the synaptic membrane. Continuous stimulation of TRPV1 receptors results in increased calcium ion concentration (Fig. 3). This increased calcium ion concentration causes continuous fusion of vesicles onto synaptic membrane thus increasing surface expression, which leads to thermal hyperalgesia.

Role of KIF1B and KIF5A in Charcot-Marie-Tooth Disorder and Hereditary Motor and Sensory Neuropathy

Charcot-Marie-Tooth (CMT) disorder and hereditary motor and sensory neuropathy (HMSN) are a spectrum of diseases that are due to a mutation in genes related to myelin sheath and axonal length [54]. It is predominantly autosomal dominant, but sometimes it can be autosomal recessive (for example CMT 4) [55]. In most cases it is inherited, but some cases there are non-inherited spontaneous errors when DNA was being formed that are called sporadic. Genetics plays an

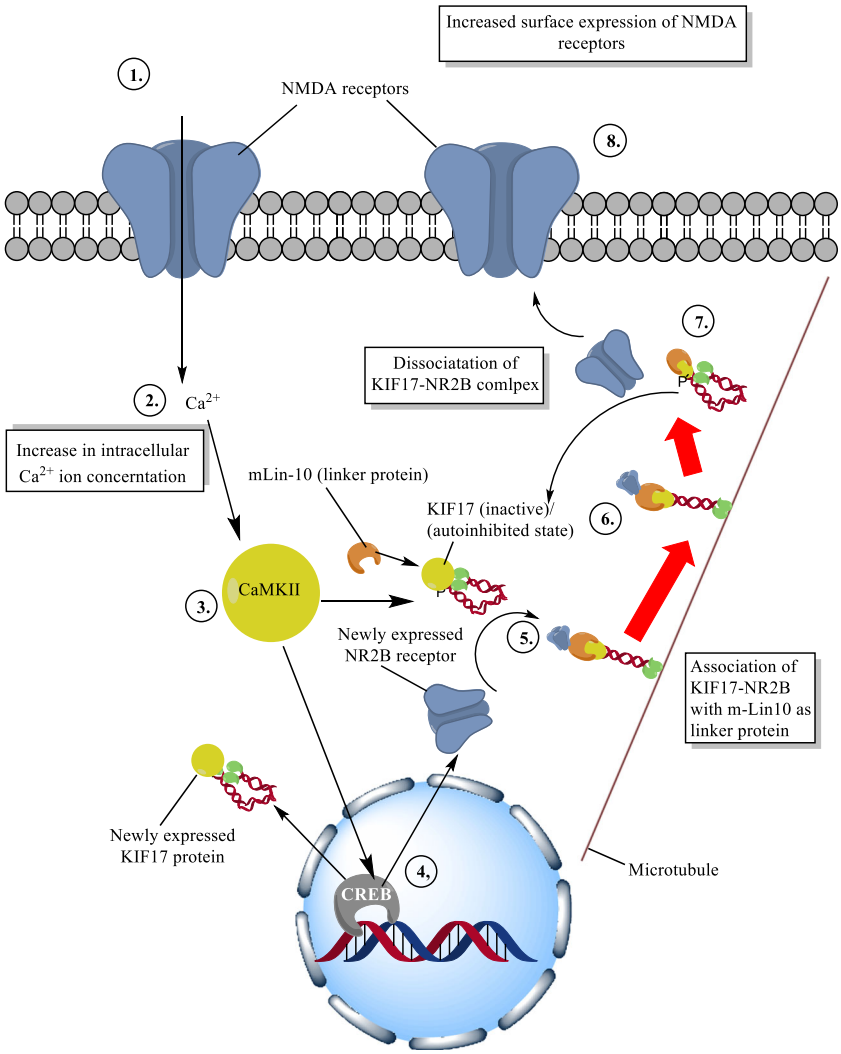

Fig. 1 KIF17 mediated transport of NR2B receptors to synaptic membrane. NMDA receptors (NR2B receptors) present on the dorsal root ganglion (DRG) and spinal cord (SC) neurons gets stimulated on receiving the stimulus from peripheral neurons. This causes activation of Ca^{2+}/ calmodulin-dependent protein kinase II (CaMKII) via increased calcium influx. CaMKII act on CREB (cAMP response element binding) protein and stimulates synthesis of NR2B, KIF17 and also activates KIF17 in the cytoplasm. Newly synthesized NR2B receptor gets associated with KIF17 with the help of Mlin-10 linker protein. Afterwards, this protein complex binds to microtubule and delivers it to synaptic membrane

important role in diagnosis whether the problem is in myelin sheath or axon. This leads to muscle and bone deformities. Increased muscle wasting and thinning of the leg is observed as the muscle gets atrophied. Decrease in muscle mass makes sensory neurons more prone to compression leading to paresthesia. There is an increase in the threshold of pain if spinothalamic tract fibers become involved, and sense of proprioception is decreased. If there is reduction in response relative to the speed of conduction, then CMT will be classified as axonal form. If the speed of conduction is slowed with relative preservation of the amplitude of response, then it is classified as demyelinating form. However combination of both can also be present, particularly in late stages of disease, which makes diagnosis and treatment confusing.

CMT has one to seven types and occurs mainly due to mutation in four genes PMP22 (Peripheral Myelin Protein 22), GJB1 (Gap junction beta-1 protein) also called connexin 32, MPZ1 (Multi-PDZ Domain-containing Protein-1), and GDAP1 (Ganglioside-induced differentiation-associated protein 1). CMT 1 is a predominately demyelinating form, with autosomal dominant form CMT 1A, which is due to a mutation in PMP22 gene present in chromosome 17, leading to dysfunction in the formation of the myelin sheath. Another form is CMT1B which has an error in gene encoding MPZ, this disease tends to present early in age and is more severe. CMT 2 is an autosomal dominant axonal form that is rarely recessive. The extent of severity depends upon encoding gene. In a study conducted by Manganelli et al. [56], the genetic diagnosis of CMT was confirmed in 145 cases out of 197 in Campania, Southern Italy. In this study, it was observed that mutation in four genes (PMP22, GJB1, MPZ1, and GDAP1) was associated with 92% of genetically confirmed cases. CMT 1 mutation is mainly due to PMP22, MPZ, and SH3TC2. Mutation in GJB1 and GDAP1 resulted in CMT 2 patients. This was observed in more than three-fourth cases of CMT 2 patients.

Mutation in genes encoding for KIF1Bβ and KIF5A is known to be associated with type 2 CMT [57]. To confirm

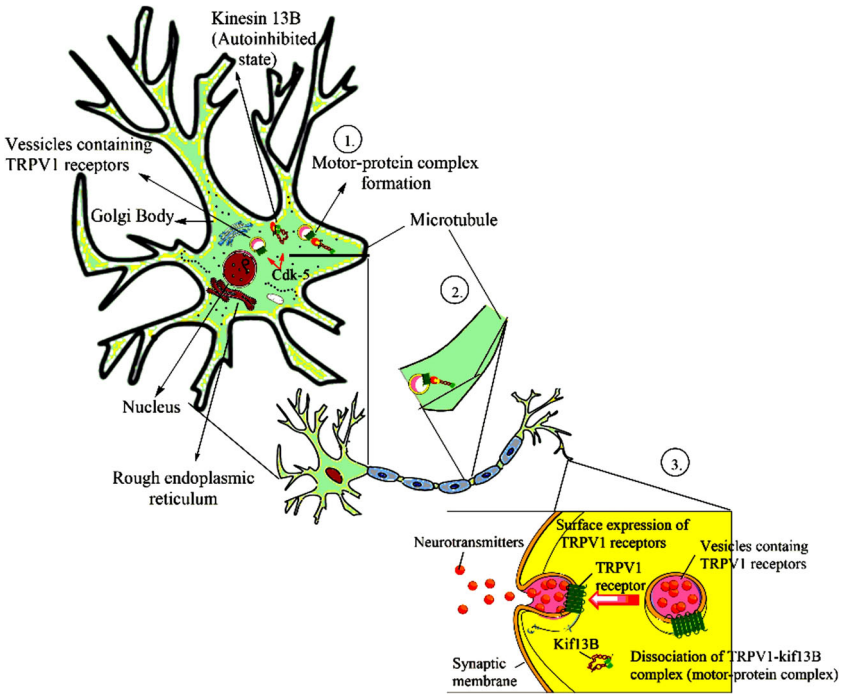

Fig. 2 Mechanism of transport of TRPV1 receptors across the neuron by KIF13B. (1) TRPV1 receptors are synthesized by the nucleus and packed into vesicles by the Golgi body. After this, vesicle forms association with KIF13B by possible linker protein. This KIF13B-TRPV1 complex (motor-cargo) association is facilitated by the phosphorylation of both TRPV1 and KIF13B by Cyclin-Dependent Kinase-5 (CDK-5). (2) KIF13B-TRPV1 complex binds to microtubule and moves along microtubule to transport TRPV1 receptors across the axon. (3) On reaching synapse membrane, this KIF13B-TRPV1 complex dissociates to release the vesicle. The dissociated vesicles are now docked onto the synaptic membrane. This docked vesicle fuses with synaptic membrane thus expressing TRPV1 receptors on the surface of neurons. Continuous expression of TRPV1 receptors on the synaptic membrane results in thermal hyperalgesia

the involvement of the KIF5A in type 2 CMT and to define the frequency of KIF5A mutations in an Italian hereditary spastic paraplegia (HSP) population, authors performed the genetic screening in a series of 36 CMT2 and 139 HSP affected subjects. They found total five mutations, one in a CMT2 subject and four in HSP patients. All the five mutations are localized in the kinesin motor domain except for one which was found in stalk domain and found to be involved in protein structure destabilization. The findings suggested a KIF5A mutation frequency of 8.8% in the Italian HSP population and identify the stalk domain, a region of the kinesin protein, as novel target for mutation. Mutation of KIF5A is also known to be involved in the HSP. In another study, López et al. [58] identified two novel KIF5A mutations (c.773 and c.833) in Spanish patients suffering from hereditary spastic paraplegia with mild peripheral neuropathy. Both the mutations were located in the highly conserved kinesin motor domain which was identified as hot spot for KIF5A mutations. These findings adds to the evidence suggesting occurrence of mild peripheral neuropathy in the adult onset HSP [58].

Role of KIF5B in Pain: Modulation of Sodium Ion Channels

Involvement of sodium channels in pain is well explored. It has several isoforms Na_v 1.1 to 1.9. Three specific isoforms, Na_v 1.7, Na_v 1.8, and Na_v 1.9, which are expressed in peripheral nervous system, are predominantly responsible for pain perception [59]. In peripheral inflammation of the sciatic nerve, the expression of Na_v 1.8 is increased [60] and kinesin 1 is reported to be involved in the anterograde transport of sodium ion channels [61].

A study by Su et al. [37] suggested KIF5B involvement in anterograde transport of Na_v 1.8. Authors used CFA-induced inflammatory pain model and injected 200 μl of CFA into the hind paw. They used shRNAs of KIF5A, KIF5B, and KIF5C to knock out expression of all isoforms of kinesin 1 one by one in cultured DRG neurons and transfected ND1-23 and HEK293T cell lines. Findings suggested that knocking out KIF5B leads to decreased surface expression of Na_v 1.8 [37].

Conclusions and Future Perspectives

Targeting kinesin for the treatment of pain is a novel strategy. Kinesins have been well explored as a target for cancer therapy but it is only until recently when scientists have investigated their potential role in pain regulation. In cancer, there is increased expression of proteins, receptors, and their associated kinesin, so to prevent the overexpression of receptors and to decrease the responsiveness to the growth stimulus, kinesin inhibitors are used [62]. A similar overexpression of kinesins

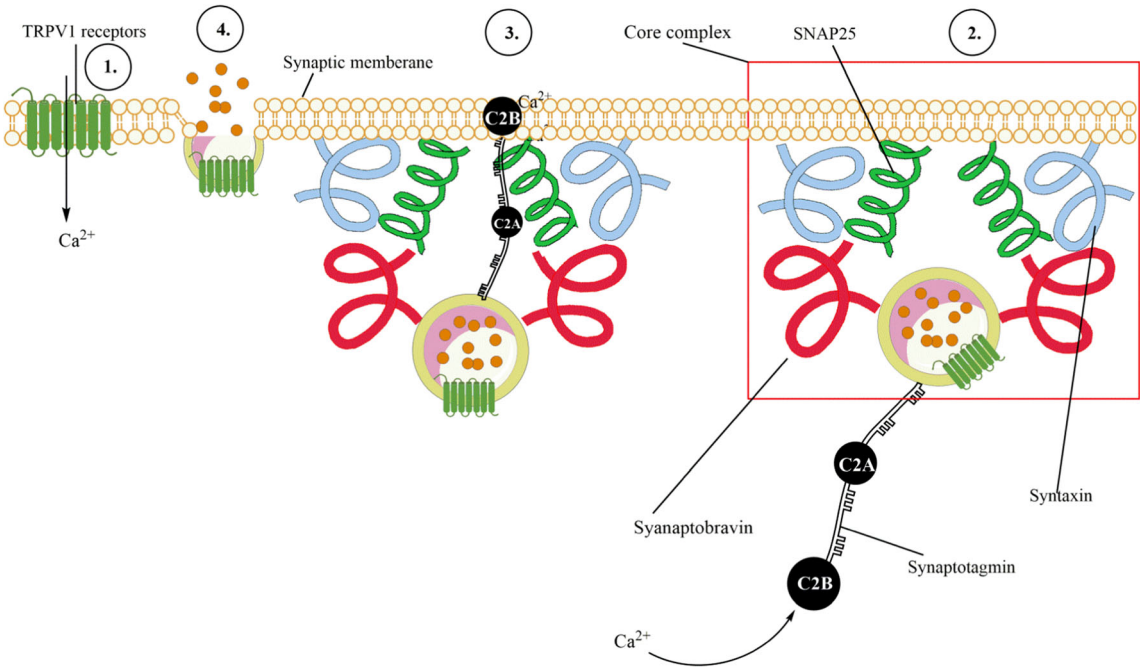

Fig. 3 Mechanism of surface expression of TRPV1 receptors. (1) The vesicle containing TRPV1 receptors are transported by KIF13B to the synaptic membrane. Some TRPV1 already present on the synaptic membrane get stimulated and increase calcium ions influx. (2) The TRPV1 containing vesicles which are docked onto the synaptic membrane by syntaxin, synaptobrevin, and SNAP-25, the calcium ions come and bind to a C2B domain of synaptotagmin. (3) C2A binds to syntaxin whereas C2B-Ca^{2+} domain causes disturbance in the phospholipid bilayer resulting a fusion of the vesicle with the synaptic membrane. (4) This fusion results in the release of contents inside the vesicles (e.g., neurotransmitters) and TRPV1 receptors get expressed onto the surface of the synaptic membrane. Due continuous stimulation of TRPV1 receptors results in increased calcium ion concentration. This increased calcium ion concentration causes continuous fusion of vesicles onto synaptic membrane thus increasing surface expression. This increased surface expression of TRPV1 receptors leads to thermal hyperalgesia

and receptors responsible for nociception can be observed in case of pain such as overexpression of CaMKII, KIF 17, and NR2B during bone cancer pain. This was further supported on using KN93 (CaMKII inhibitor) and antisense ODNs which were found to attenuate pain [39, 43]. In case of CFA-induced inflammatory pain, CDK-5, KIF 13B, TRPV1, and Na_v 1.8 were found to be overexpressed [37, 44]. Roscovitine, a CDK-5 inhibitor and shRNA against KIF 5B, having a purine core potentially relieved pain.

The use of kinesin inhibitors, or the inhibitor of regulatory protein/enzyme associated with kinesin, decreases the transport of proteins across the axons as KIF17, mLin-10 (linker protein), and CaMKII are involved in the transport of NR2B in bone cancer pain. Inhibitor of KIF17, mLin-10, or CaMKII decreases the mechanical allodynia and thermal hyperalgesia but has the possibility of impairing the learning and memory [63]. Use of CDK-5 inhibitors in the treatment of pain has the possibility of impairing cell division in other dividing cells, and the cells of hair follicles, nail, and bone marrow will be preferentially affected. Kinesins can sometimes be non-specific in the transportation of cargos. Inhibition of such kinesins in the absence of such cargos can lead to potentially deleterious effects, for example, KIF 5 inhibition can affect transit of both sodium channels and GABA receptors, though reduction of sodium channels causes pain attenuation, but loss of GABA may lead to potential side effects [38]. There is still a lot to be explored in this field; the linker proteins that are involved in the formation of motor-cargo complex in KIF 13B are not yet identified. This linker protein and inhibition of these linker proteins may result in pain relief. There are still lot of kinase enzymes which are involved in the activation of kinesins which needs to be discovered and the effects influence preprocessing of tubulin and pain need to be explored further. Finally, the late regulatory events that govern the release of receptors need to be discovered. Influencing these factors in a targeted way may help in the specific modulation of pain pathways and may lead to the development of novel pain therapies with better safety and tolerability profile. Apart from this, inhibitors for kinesin or enzymes responsible for kinesin activations could also be developed or repurposed as an alternative, safe, and potential therapy for the treatment of chronic pain.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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