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TOPICAL REVIEW

SMURF and NEDD4: Sharp Shooters Monitor the Gate Keepers and Ion Traffic Controllers of Lead Astray Cell

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Abstract It is becoming increasingly apparent that a complex bar code underlies the quantitative aspects of extracellular signal regulation. Cell type-specific and context-dependent transcriptional programs are triggered by sophisticated nanomachinery consisting of HECT enzymes which monitor signal generation, transduction and termination. How the HECT enzymes safeguard spatiotemporal organization was a fundamental question towards understanding the process of protein degradation and its functions in diverse biological processes. In this review we will dismantle how HECT E3 enzymes regulate the trafficking of many receptors, channels and transporters as well as how HECT enzymes negatively regulate each other. There is accumulating evidence that suggests an undeniable role of HECT enzymes in regulating mediators of the Wnt signal-transduction cascade. By contrast, little is known about the crosstalk of HECT enzymes with ATM and TRAIL in prostate cancer, but several hints have emerged. This review provides a broader snapshot for studying multiple pathways in parallel, rather than as separate entities.

Keywords SMURF · NEDD4 · Prostate cancer

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Introduction

Numerous signals generated by the ubiquitin system have been implicated in the central regulation of biological processes and transmission of information in the cell. Substantial understanding has been gained about the fundamental role of ubiquitin species of diverse lengths and linkages in the endocytic trafficking of channels and protein degradation. This harmonized and well-coordinated surveillance is achieved through complicated mechanisms of compartmentalization and a sequential series of ubiquitylation events and signal decoding.

It is worth mentioning that ubiquitylation is triggered by an orchestrated action of activating (E1), conjugating (E2) and ligating (E3) enzymes, several of which have elongating activities that modulate the generation of polyubiquitin chains. Accumulating evidence suggests that this specific posttranslational modification occurs primarily through the conjugation of monoubiquitin or polyubiquitin chains of variable length on any of the seven Lys residues (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48 or Lys63) or the amino-terminal Met (Met1) of the ubiquitin monomer. Ubiquitin is nonetheless a very conserved polypeptide of 76 amino acids. Recent data suggest that a large number of ubiquitin signal species are recognized and decoded by specialized ubiquitin-binding domains (UBDs), which trigger transitory, noncovalent interactions with either ubiquitin moieties or hallmark linkage regions residing in ubiquitin chains. Ubiquitin contains a diglycine motif at its COOH-terminal end; furthermore, it is ligated through this COOH terminus to lysine residues of proteins destined for degradation. Details can be found elsewhere (Grabbe et al. 2011).

The transforming growth factor (TGF)-β superfamily has broad implications in terms of cell proliferation, apoptosis, differentiation, migration and development. It is

a matter of great concern that the intensity of receptor-mediated cellular signaling must lie within a precise and accurate range. Any insufficient signaling can lead to developmental disorders or tissue atrophy; paradoxically, over-signaling can lead to hyperplastic and eventually neoplastic events. In the following sections we will emphasize the disruption in spatiotemporal organization of negative regulators of the TGF signaling cascade which leads to serious molecular anomalies. Figure 2b shows an overview of the internalization of the TGF receptor (TGFR) and interaction with SMURF and NEDD4.

Encounter of Traffic Cops and Trouble Mongers: SMURF Faces Tit for Tat

It is well recognized that the ubiquitin–proteasome system degrades a huge multiplicity of proteins that contain specific degradation signals, or "degrons." Structural and functional information on the ubiquitin ligases which target the proteins for degradation or for other cellular fates allows several generalizations regarding their mechanism of action. Figure 1 shows the structural framework of SMURF and NEDD4.

There is currently extensive confirmation for a contributory role of aberrant activity of RhoA in cellular disorders. These nanoparticles function as GDP/GTP-modulated binary switches that direct several essential cellular processes. There are various cellular disorders which are tightly interconnected with mechanism of GTPase deregulation or deregulated expression and/or activity of their regulatory proteins. In this section, we assess the association of RhoA with SMURF. Additionally, we outline some mechanisms by which SMURF1 is negatively regulated by FBXL15, REGγ and SMURF2.

It is becoming increasingly apparent that interfering with the ubiquitin binding surface (UBS) blocks SMURF-dependent degradation of its substrate RhoA and severely abrogates well-organized binding of a monoubiquitylated form of RhoA to the SMURF HECT domain in cells. Analysis of mutants of the HECT UBS through NMR spectroscopy revealed that the UBS was required for polyubiquitylation of both SMURF itself and the SMURF substrate RhoA. However, astonishingly, it was not necessary for monoubiquitylation. Therefore, it seems important to note that UBS promotes polyubiquitylation by stabilizing ubiquitylated substrate binding to the HECT domain (Ogunjimi et al. 2010). The C2 domain of SMURF1 is essential for binding RhoA and, consequently, for targeting RhoA for ubiquitination (Tian et al. 2011) (Fig. 4).

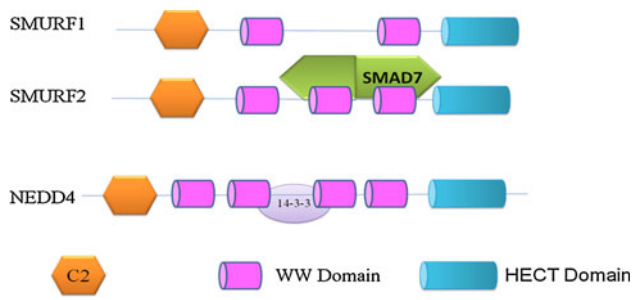

Fig. 1 Structural framework of SMURF and NEDD4

Additionally, some other domains of SMURF are important in the regulation of RhoA. In accordance with this concept, crystal structure analysis has shown that the SMURF1 C2 domain possesses a typical antiparallel β-sandwich fold. Investigation of the sulfate-binding site has revealed that two key lysine residues, Lys-28 and Lys-85, within the C2 domain are central for localization of SMURF at the cellular surface and robust ligase activity toward RhoA (Lu et al. 2011) (Fig. 4).

In order to maintain molecular stoichiometry, SMURF is negatively regulated as well. It has been documented that F-box and LRR domain-containing protein 15 (FBXL15), an F-box protein of the FBXL family, forms an Skp1-Cullin1-F-box protein-Roc1 (SCF, FBXL15) ubiquitin ligase complex and triggers ubiquitination and proteasomal degradation of SMURF. Structural studies have indicated that FBXL15, through its leucine-rich repeat domain, particularly interacts with a subdomain within the N-lobe of the SMURF1 HECT domain and facilitates the ubiquitination of SMURF1 on K355 and K357 within the WW-HECT linker region. Furthermore, ablation of FBXL15 results in stabilization of SMURF; on the contrary, enforced expression of FBXL15 results in suppression of SMURF in BMP-deficient mutants (Cui et al. 2011).

Another interesting piece of evidence is the diametrically opposed relationship of SMURF2 with SMURF1. Experimental information verified that SMURF2 interacted with SMURF1 and triggered its ubiquitination and degradation; however, discordantly, SMURF1 failed to induce degradation of SMURF2. Abrogation of SMURF2 in human breast cancer MDA-MB-231 cells resulted in a substantial rise in the levels of SMURF1 protein (Fukunaga et al. 2008) (Fig. 4).

It has recently been found that REGγ, an activator of 20S proteasome-mediated protein degradation, interacts with SMURF1 and is instrumental in its degradation. Experimental evidence shows that abrogation of REGγ or cells deficient in REGγ displayed enhanced stability of SMURF1, while reconstitution of REGγ in deficient cells retrieved degradation of SMURF1. Fascinatingly, both SMURF2 and SMURF1 were destabilized by the REGγ proteasome (Nie et al. 2010) (Fig. 4).

It has recently been found that tumor necrosis factor (TNF) receptor-associated factor-4 (TRAF4) is a new

target of SMURF1, which polyubiquitylates TRAF4 to mediate its proteasomal degradation. In a set of experiments in human embryonic kidney 293 (HEK293) cells, abrogation of SMURF1 escalated TRAF4 levels. However, these elevated levels of TRAF4 were reversed upon reconstitution of HEK293 cells (enforced expression of SMURF), demonstrating endogenous regulation of TRAF4 by SMURF1 (Kalkan et al. 2009).

Split Personality of SMURF: Dual Responsibilities as Candyman by Day and Undertaker by Night

Accumulating data underscore the ability of adaptor proteins to generate signaling platforms that modulate biologically distinct cellular transformation upon integrin-dependent adhesion and growth factor receptor activation. In this section we spotlight the significance of adaptor proteins in signaling that originates from integrin-mediated cell–extracellular matrix (ECM) adhesion and growth factor stimulation as well as Wnt-mediated signal transduction and their crosstalk with SMURF. We particularly emphasize the mechanistic insights of regulation of p130 Crk-associated substrate (p130CAS), NEDD9, effectors of Wnt signaling (β-catenin) and negative regulators of Wnt signaling (Axin, GSK beta) by SMURF in mediating cellular activities.

Evidence suggests that the epidermal growth factor receptor (EGFR) is overexpressed in a variety of cancers, and there is a synchronous activity of EGFR with SMURF2 in led astray cell as it chaperones it from degradation by c-Cbl (Ray et al. 2011).

It is well-recognized that Axin and GSK-3β negativelt regulate the Wnt/β-catenin signal-transduction cascade via regulating the level of β-catenin, which is a central effector molecule. SMURF2 degraded Axin by addition of ubiquitin moieties to Lys(505); moreover, the Axin(K505R) mutant resisted degradation. Consistent with the same analysis, knockdown of endogenous SMURF2 augmented the level of endogenous GSK-3β and resulted in suppressed β-catenin/Tcf reporter activity (Kim and Jho 2010; Wu et al. 2009) (Fig. 2a). β-Catenin also evades degradation by SMURF2 when it associates with SMAD7. Smad7 promotes the stabilized β-catenin to form a cytoplasmic signalosome with E-cadherin and stabilizes the E-cadherin–β-catenin complex to mediate cell–cell adhesion (Tang et al. 2008). Emerging evidence confirms the fact that the HECT-WW protein SMURF2 physically associates with the multifunctional adaptor protein NEDD9 and is essential for the stability of the NEDD9 protein. Cells deficient in SMURF2 have a noticeable decrease in NEDD9 protein levels. Alternatively, reintroduction of SMURF2 results in elevation of endogenous NEDD9 protein, supporting a role for SMURF2 in NEDD9 stability (Moore et al. 2010) (Fig. 3).

In the BMP pathway, GSK3 phosphorylates Smad1, severely impairs YAP-specific binding patterns and adds binding sites for SMURF1 WW domains. Correspondingly, in the TGF-β pathway, GSK3 phosphorylates Smad3, which results in addition of sites to augment affiliation for Nedd4L. It is becoming progressively more apparent that a complex bar code underlies the TGF and BMP signal-transduction cascade as Smad phosphoserine and a set of WW domain code readers regulate the spatiotemporal organization of signaling cascades (Aragón et al. 2011).

In support of this notion, emerging data verify that HECT-type ubiquitin ligase (E3) Smad ubiquitination regulatory factor 1 (SMURF1) targets a broad range of substrates, including Smad1/5, RhoA, Prickle 1, MEKK2 and JunB, for degradation. Another substrate of SMURF documented to be regulated is endoplasmic reticulum (ER)-localized Wolfram syndrome protein (WFS1). It is intriguing to note that the C-terminal luminal region in

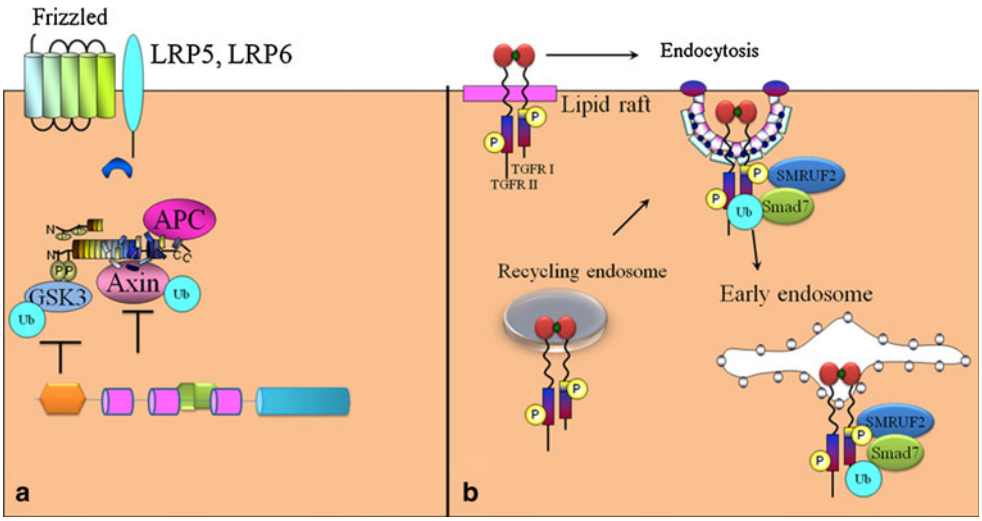

Fig. 2 **a** SMURF2-mediated degradation of negative regulators (GSK3 and Axin) of Wnt signaling. **b** Endocytosis/internalization of TGFR after addition of ubiquitin moieties

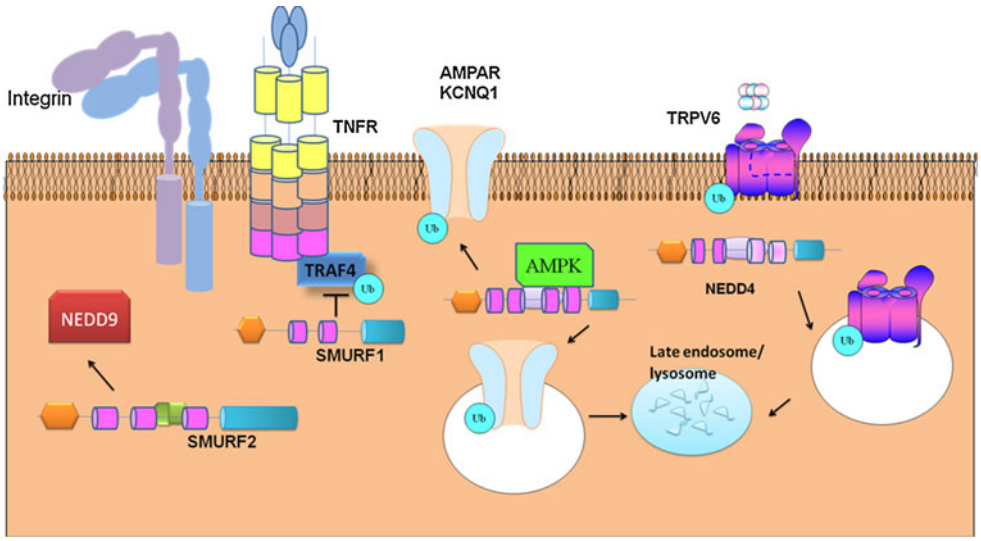

Fig. 3 Degradation of TRAF4 by SMURF2. AMPK-induced phosphorylation of NEDD4 resulted in degradation of KCNQ1 and mutant NEDD4-mediated degradation of TRPV6. However, SMURF2 stabilized protein levels of NEDD9

WFS1, encompassing a characteristic stretch of residues (degron) 667–700, is implicated in SMURF-mediated degradation. Escalating evidence suggests that both wild-type and a subset of WFS1 mutants that include this degron region are prone to SMURF1-mediated degradation; however, degron-deficient mutants of WFS1, such as W648X, Y660X and Q667X, evade degradation by SMURF1. Similarly, attenuation of SMURF1 results in increased WFS1 and reestablishment of SMURF1 results in suppression of WFS1 (Guo et al. 2011). Using protein microarray for assaying ubiquitination with SMURF1 and the partner E2 ubiquitin ligase Ubch5 or Ubch7, it has recently been shown that microarray-derived data identified 89 potential substrates of SMURF1 E3 activity, which spanned a number of different biological pathways (Andrews et al. 2010).

NEDD4: The Master Regulator of "Border Guards"

The never-ending trafficking of ions across cell membranes is regulated by two kinds of border guards: ion channels and ion pumps. Open channels allow selected ions to diffuse rapidly down specific transient electrical and concentration gradients. On the contrary, ion pumps are major determinants for maintenance of the gradients by consuming energy to slowly move ions thermodynamically uphill. In this section we discuss the tight interaction between channels and SMURF/NEDD4 as structural and mechanistic information about these particular molecular machines has improved and been refined considerably.

We also highlight Nedd4 and Nedd4-2 ubiquitin ligases and the mechanisms by which they adjust ion channels and portray a novel exemplar of the mechanisms that underpin anomalous ion channel functions in multiple molecular disorders.

Lately, a consequence of coexpression of Nedd4-2 on the apical Ca^{2+} channel TRPV6, which is involved in transcellular Ca^{2+} transport, was examined. The HECT domain is vital to the inhibitory effect of Nedd4-2 on TRPV6 and to their association. WW1 and WW2 domains interacted with TRPV6 terminal regions, and interruptions in the associations by mutations in the WW1 and WW2 domains augmented TRPV6 ubiquitination and degradation. Thus, wild-type WW1 and WW2 domains of NEDD act as a molecular switch to restrict the ubiquitination of TRPV6 by the HECT domain (Zhang et al. 2010) (Fig. 3). Future investigations must characterize mutations in the HECT and WW1/2 domains in prostate cancer as disruption of the reciprocal relation between two domains determines the extent of protein degradation. Furthermore, there is a possibility that loss-of-function mutations in the WW1 and WW2 domains results in facilitating the degradation of proteins. It has recently been demonstrated by surface biotinylation assays that Nedd4-1 decreased the number of channels inserted at the plasma membrane. Nedd4-1 triggers newly synthesized Cav channels for proteasomal and lysosomal degradation. In this molecular mechanism NEDD4-1 is often challenged by the cavβ-subunit, which is involved in catapulting channels from the ER to plasma membrane (Rougier et al. 2011).

Another exciting piece of evidence in the tight correlation between NEDD4 and membrane channels is exemplified by AMPA receptors (AMPARs). AMPARs are the key players involved in excitatory synaptic transmission in the brain. Ubiquitination enhances AMPAR endocytosis, as a result of which AMPAR cell-surface localization and total receptor abundance undergo a reduction. It is also documented that certain amino acid residues are important in regulating the mechanism of ubiquitination. For instance, mutation of lysine residues to arginine residues at

the GluA1 C terminus considerably reduces GluA1 ubiquitination and remarkably hampers ubiquitin-dependent GluA1 internalization and degradation. It was also found that synaptosomes were richly supplied with Nedd4 and, thus, negatively regulate AMPARs in neurons. On the contrary, abrogation of Nedd4 suppresses AMPAR ubiquitination (Lin et al. 2011). Another point of attraction is that ubiquitination of GluA1 by Nedd4-1 becomes more prevalent in parallel to neuron maturation. Collectively, it is obvious that ubiquitination of GluA1-containing AMPARs by Nedd4-1 triggers their endocytosis and shuttling to the lysosome (Schwarz et al. 2010).

It is essential to note that mutations in NEDD4 disrupt binding affiliations of NEDD4 with KCNQ and Kir2.1 channels and, consequently, escape of channels from degradation and decrease in channel-mediated currents, respectively. This therefore provides a model for the investigation of the "safety margin" that demarcates normal from pathological levels of channel expression. Mounting evidence suggests that wild-type NEDD4 is phosphorylated by AMP-activated protein kinase (AMPK) and noticeably enhanced Kir2.1-mediated currents and Kir2.1 protein abundance in the cell membrane. Additionally, AMPK partially triggers Kir2.1-mediated currents via phosphorylating NEDD4-2. Discordantly, Nedd4-2(S795A) lacking an AMPK phosphorylation consensus sequence severely compromised Kir2.1-mediated currents (Alesutan et al. 2011b). It is a well-established fact that Nedd4 ubiquitin ligases downregulate both potassium and sodium channels. In accordance with the same assumption, heterotetrameric K^+-channel KCNQ1/KCNE1 currents and KCNQ1 protein abundance in the cell membrane were substantially decreased by Nedd4-2 (Alesutan et al. 2011a). Another intriguing piece of information is that AMPK inhibits KCNQ1 activity by enhancing Nedd4-2-dependent channel ubiquitination and reclamation from the plasma membrane (Alzamora et al. 2010) (Fig. 3).

Ubiquitination mediated by NEDD4-2 or NEDD4-1 is doubtlessly the common means of regulation of various trasporter proteins by protein kinase C (PKC). The mechanism by which PKC exerts its effects via NEDD4-1/2 was evaluated by interference of NEDD4-1/2 in HEK293 cells where cationic amino acid transporter 1 (CAT-1) escaped ubiquitination despite the presence of PKC. Furthermore, overexpression of Nedd4-2 recapitulated PKC-dependent ubiquitination of dopamine transporter (DAT). (Vina-Vilaseca et al. 2011; Vina-Vilaseca and Sorkin 2010). The epithelial Na^+ channel (ENaC) is a vital mediator in the maintenance of sodium balance and consequently of blood pressure. It is modulated by multiple signaling pathways together with ubiquitylation via the ubiquitin-protein ligase NEDD4-2. It is documented that ENaC PY motifs offer the binding sites for NEDD4-2 (Ruffieux-Daidié and Staub 2011). It has recently been shown that ENaC interacts with hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs), a component of the endosomal sorting complexes required for transport (ESCRT)-0 complex. These interactions are enhanced by NEDD4-2, which plays a dominant role in establishing an association between these two proteins (Zhou et al. 2010).

However, various kinases chaperone ENaC from degradation by NEDD4-2. Importantly, SGK1 (serum- and glucocorticoid-inducible kinase type 1) interacts with WW domains 2 and 3 of Nedd4-2 and PKBα enhances ENaC surface expression by phosphorylating Nedd4-2, thereby preventing ENaC internalization and degradation (Diakov et al. 2010; Wiemuth et al. 2010). Interestingly, manganese upregulates the expression of NEDD4-2 and downregulates SGK1 to suppress SNAT3, a key protein involved in glutamine (Gln) efflux from astrocytes (Sidoryk-Wegrzynowicz et al. 2010). Consistent with the same concept, it is also clear that stimulation of peroxisome proliferator-activated receptor gamma (PPARγ) results in a higher activity of ENaC by a decrease in ubiquitination through the SGK1/Nedd4-2 pathway (Renauld et al. 2010). The interpretations are poorly elucidated, and future evaluations are necessary to have a broader view of the crosstalk of SGK1 and Nedd4-2. Evidence suggests that WNK (with no lysine [K]) protein kinases are involved in the activation of SGK1. It is also noteworthy that Nedd4-2 couples to WNK1 and endogenous SGK1 has reduced activity if WNK1 is knocked down by small interfering RNA (Heise et al. 2010). It has yet to be explored whether WNK/NEDD4-2 interaction is engaged in reducing the affiliation for ENaC or if it potentiates the inhibitory effects of SGK1. It is indicated from several lines of evidence that copper metabolism Murr1 domain-containing protein 1 (COMMD1, previously known as Murr1), a protein specific for copper metabolism, repressed currents in *Xenopus laevis* oocytes expressing ENaC. Data suggested that COMMD1 had suppressive effects on SGK1 and that impairment of COMMD1 resulted in elevation of SGK1 activities. There is another documented relation of COMMD1 with NEDD4-2 in the inhibition of ENaC cell-surface expression (Ke et al. 2010).

Compelling evidence suggests that sodium channels undergo a reduction in current density after biochemical modifications triggered by p38 MAPK and Nedd4-2 (Gasser et al. 2010). However, interestingly, NEDD4 promotes the expression of SNAT3 and their affinity and subsequent hyperubiquitination of SNAT3 are noticeably enhanced upon PKC activation (Sidoryk-Wegrzynowicz et al. 2011).

Recently, it was found that NEDD4-1 undergoes post-translational modification as O-GlcNAc modification is the first reported glycosylation of this protein (Zaro et al. 2011). It is not known whether NEDD4-2 shows opposing activity trends. It is well known as a mechanism of ubiquitylation;

however, it also facilitates deubiquitylation. In support of this notion, an exciting piece of evidence is that both the catalytic and N-terminal tails of Usp2-45 physically interact with the HECT domain of Nedd4-2. This interaction is necessary to allow juxtapositioning of Usp2-45 to ENaC for its deubiquitylation (Oberfeld et al. 2011).

It is noteworthy that inwardly rectifying K^+ (Kir) channels have numerous functions, including the complicated regulation of neuronal signaling. Importantly, K^+ channels allow K^+ flux and are vital for the generation of electric current across excitable membranes. Since the physiological significance of these channels cannot be overlooked, a substantial attempt has been made to improve and clarify our understanding of the structural origin of their physiology. However, recently unfolding high-resolution structures and intricate crosstalk between K^+ channels and NEDD4 have opened new avenues for the evaluation of inward rectification and their regulation by intracellular factors.

It is intriguing to note that G protein-coupled receptor kinase (GRK2) is a family member of the protein kinases and contains a regulator of the G protein-signaling homology (RH) domain, which differentially associates with α-subunits of the Gq/11 family that are detached during G protein-coupled receptor activation. It is known that kinase activity of GRK2 robustly enhances ENaC activity by blocking Nedd4-2-dependent inhibition of ENaC. Another piece of information that refines GRK2-mediated ENaC signaling is that GRK2 modulates the activity of ENaC via Gαq/11. In a set of experiments it was clearly revealed that wild-type GRK2 and a kinase-dead GRK2 mutant remarkably enhanced activity of ENaC. On the contrary, a GRK2 mutant that lacks the C-terminal RH domain and a GRK2 mutant that cannot interact with Gαq/11/14 suppressed GRK2-mediated ENaC activity (Lee et al. 2011).

It is well-known that voltage-gated potassium channels are sophisticated membrane signaling proteins. Structural insight into these proteins indicates that these nanomachines have pores that pass millions of ions per second across the membrane with remarkable selectivity. Intriguingly, their gates snap open and shut in milliseconds as they sense variations or transitions in voltage or ligand concentration. Recent findings have revealed interactions of voltage-gated potassium channel architectural modules with NEDD4, but some important links remain to be elucidated.

It is remarkable that improper channel localization triggered by NEDD4-2 could cause communication defects in a neuronal network. The next section covers recent studies unfolding mechanistic details for NEDD4-2-mediated targeting of voltage-gated ion channels and resulting disruption in preferential localization of ion channels in the plasma membrane. NEDD4-2 regulates hERG1 (human ether-à-go go-related gene 1) by the use of a PY motif residing in the C terminus of hERG1. This domain is indispensible for the interaction and consequent ubiquitylation of hERG as the hERG1 mutant for this motif was not ubiquitylated by NEDD4-2 (Albesa et al. 2011) (Fig. 4). Other than its well-established hallmark feature of protein degradation, it also triggers shuttling of proteins from nucleus to cytoplasm. Nedd4-1 is involved in enhancing transmigration of hDCNL1 after monoubiquitination (Wu et al. 2011).

Evidence suggests that NEDD4L is a candidate gene implicated in mediating sodium lithium countertransport (SLC) activity. Variations in the NEDD4L gene are associated with SLC activity as it is an intermediate phenotype of essential hypertension (Zheng et al. 2011).

SMURF and NEDD4 in Prostate Cancer: Paradigm in Parallax

Escalating evidence suggests that Nedd4L expression undergoes downregulation in prostate cancer compared to benign prostatic hyperplasia (Hu et al. 2009). In concordance with the same approach, it was observed that SGK1 is an androgen-regulated gene that is instrumental in prostate cancer progression. It was observed that androgen

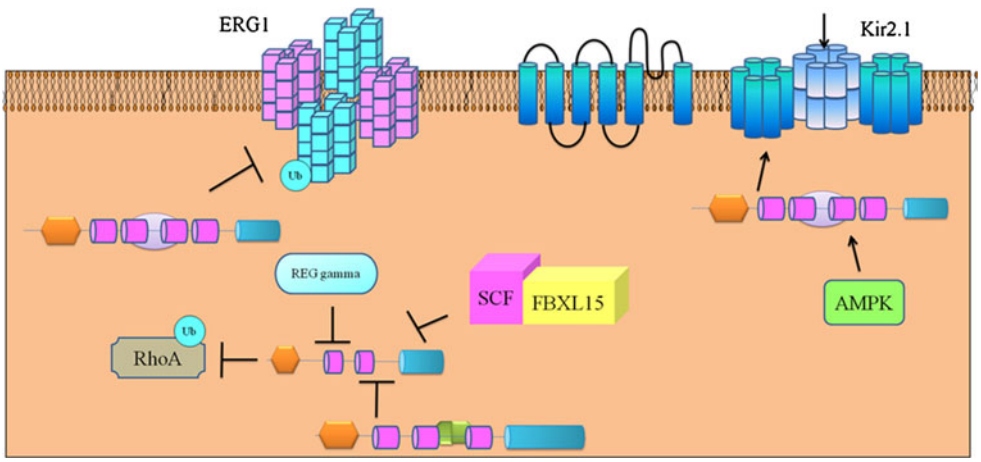

Fig. 4 Inhibition of SMURF1 by REGγ, SCF/FBXL15 and SMURF2. SMURF1 mediates degradation of RhoA. NEDD4 enhanced ubiquitylation of ERG1. NEDD4 is phosphorylated by AMPK and consequently enhances inward trafficking of ions across Kir2.1

treatment in an LNCaP cell line resulted in enhanced phosphorylation of NEDD4-2 by SGK1. On the contrary, abrogation of SGK1 resulted in a decrease in prostate cancer progression (Sherk et al. 2008). It is also well known that NEDD4 is an androgen-regulated gene. There are various isoforms of NEDD4 which are switched on upon treatment with androgen (Qi et al. 2003). Consistent with the same line, it is also noticeable that androgen receptor quantity control is primarily regulated by NEDD4. In a set of experiments it was observed that NEDD4 ubiquitinates androgen receptor via PMEPA1 (a highly androgen-inducible gene) which is a binding partner of NEDD4. It is established that PMEPA1 protein through its PY motifs interacts with the WW domains of the human NEDD4 protein. Henceforth, PMEPA and NEDD4 are appraised as negative regulators of prostate cancer (Li et al. 2008; Xu et al. 2003). Our lab has discordant findings in terms of the classical role of SMURF and NEDD4. We have shown that SMURF and NEDD4 are negative regulators of the TGF signal-transduction cascade, which has antineoplastic activity in prostate cancer. While working on the androgen-sensitive prostate cancer cell line LNCaP, we showed that SMURF and NEDD4 abrogation potentiated TGF-mediated antineoplastic activity and simultaneously suppressed genomic instability by activation of DNA repair proteins. Furthermore, ablation of SMURF and NEDD4 resulted in the suppression of FLIP (a protein that negatively regulates apoptosis) and TRAIL (a positive regulator of apoptosis) was upregulated. This was further confirmed by enforced expression of SMURF and NEDD4 that restored FLIP levels, indicating a cancer-fostering role of SMURF and NEDD4 (Farooqi et al. 2011a, b).

Conclusion

Keeping in view the unavoidable role of negative regulators of TGF signaling in protein degradation-based receptor quantity-control mechanisms, loss of such mechanisms could facilitate the onset or progression of molecular disorders. It is understandable that the regulation and biological output of these E3 s is such a multifaceted procedure. Henceforth, study of the features of these E3 s in cancer development poses some challenges which have to be addressed to get a step closer to individualized medicine.

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