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THE ASSOCIATION OF NHERF ADAPTOR PROTEINS WITH G PROTEIN–COUPLED RECEPTORS AND RECEPTOR TYROSINE KINASES*

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■ **Abstract** The sodium-hydrogen exchanger regulatory factors (NHERF-1 and NHERF-2) are a family of adaptor proteins characterized by the presence of two tandem PDZ protein interaction domains and a C-terminal domain that binds the cytoskeleton proteins ezrin, radixin, moesin, and merlin. The NHERF proteins are highly expressed in the kidney, small intestine, and other organs, where they associate with a number of transporters and ion channels, signaling proteins, and transcription factors. Recent evidence has revealed important associations between the NHERF proteins and several G protein–coupled receptors such as the β_2-adrenergic receptor, the κ-opioid receptor, and the parathyroid hormone receptor, as well as growth factor tyrosine kinase receptors such as the platelet-derived growth factor receptor and the epidermal growth factor receptor. This review summarizes the emerging data on the biochemical mechanisms, physiologic outcomes, and potential clinical implications of the assembly and disassembly of receptor/NHERF complexes.

INTRODUCTION

The sodium-hydrogen exchanger regulatory factors NHERF-1 (also known as EBP50) and NHERF-2 (also known as E3KARP) represent a family of adaptor proteins characterized by two tandem PSD-95/Drosophila discs large/ZO-1 (PDZ) protein interaction domains and a C-terminal domain that binds the ezrin-radixin-moesin-merlin (ERM) family of cytoskeletal proteins (1–4). The NHERF proteins were the first PDZ proteins found to be localized to the apical membranes of renal epithelial cells. These proteins were initially characterized as facilitating the formation of a multiprotein complex that mediated protein kinase A phosphorylation of the renal sodium-hydrogen exchanger 3 (NHE3) and the downregulation of its activity (5–9). NHERF-1 and NHERF-2 form homo- and heterodimers and also bind to other PDZ proteins to form an extended membrane/submembrane scaffold that in turn binds an array of transmembrane and soluble proteins, including other transporters and ion channels, signaling proteins, transcription factors, and cellular structural proteins (10–14).

This review focuses on a new class of NHERF targets, namely G protein–coupled receptors (GPCRs) and growth factor receptor tyrosine kinases. A growing body of evidence indicates that the NHERF proteins regulate the localization and function of several GPCRs, including the β_2-adrenergic receptor, the κ-opioid receptor, and the parathyroid hormone (PTH) receptor (15–18). NHERF proteins also interact with selected growth factor receptors such as the platelet-derived growth factor receptor (PDGFR) and the epidermal growth factor receptor (EGFR) to modulate mitogenic signaling by these receptor tyrosine kinases (19–20). Table 1 lists the membrane receptors currently known to associate with the NHERF proteins.

TABLE 1 Hormone receptor targets of NHERF*

G protein–coupled receptors
β_2-adrenergic receptor (15, 21)
κ-opioid receptor (18)
Purinergic P2Y1 receptor (21)
Parathyroid hormone 1 receptor (16)
Adenosine 2b receptor (52)
Lysophosphatidic acid receptor A2 (53)
Luteinizing hormone receptor (54)
RAMP3 of the adrenomedullin receptor (55)
Tyrosine receptor kinases
Platelet-derived growth factor receptor (19)
Epidermal growth factor receptor (20)

*References are indicated in parentheses.

This review summarizes data pointing to the role of the NHERF proteins in regulating selected hormone and growth factor receptors. It also outlines the underlying biochemical mechanisms, the physiologic outcomes, and potential clinical implications of the dynamic assembly and disassembly of known receptor/NHERF complexes. By highlighting common themes, we hope this review provides new insights in hormone signaling as well as direction for future studies of cell signaling by membrane receptor complexes.

G PROTEIN–COUPLED RECEPTORS AS TARGETS OF NHERF

NHERF Associates with the β_2-Adrenergic Receptor

The first receptor found to associate with the NHERF proteins was the β_2-adrenergic receptor (β_2-AR) (15). This interaction was discovered using a biochemical purification approach in which the β_2-AR C terminus was expressed as a fusion protein, linked to an agarose matrix, and used to purify interacting proteins from crude tissue extracts. A single 50 kDa protein was purified from kidney lysates, and amino sequencing revealed this protein to be NHERF-1. Mutagenesis studies demonstrated that the last four amino acids (D-S-L-L) of the β_2-AR C terminus are critical for the association of β_2-AR with NHERF-1, with the serine at the –2 position and the terminal leucine residue being of particular importance (15, 21). NHE3, the first identified NHERF target, had been shown to bind to PDZ-II of NHERF-1 (22). β_2-AR, by contrast, was the first of now a large number of polypeptides found to bind to PDZ-I of NHERF-1 (15). β_2-AR also binds to the first PDZ domain of NHERF-2 (21).

NHERF-1 Regulates β_2-AR Trafficking

The NHERF proteins play an important role in the regulation of β_2-AR trafficking. The association between β_2-AR and NHERF-1 occurs in an agonist-promoted manner. Agonist-bound wild-type β_2-AR normally recycles to the plasma membrane; this process requires NHERF-1. Introduction of mutations in the NHERF-binding motif of β_2-AR resulted in agonist-promoted internalization of the receptor and shunting of β_2-AR to lysosomes, a pathway leading to degradation of the receptor (23). Overexpression of the first PDZ domain of NHERF-1, which disrupts the association of β_2-AR with endogenous NHERF, altered β_2-AR trafficking in a fashion similar to the effect of mutation of the receptor's NHERF-binding motif (23). Furthermore, fusion of the NHERF-binding motif to other GPCRs, such as the δ-opioid receptor, enhances receptor recycling to the membrane following agonist-induced endocytosis (24, 25). These findings provide strong evidence that the NHERF proteins play a pivotal role in directing β_2-AR endocytic sorting. A regulated mechanism controlling the interaction between β_2-AR and NHERF-1 was suggested by the observation that G protein–coupled receptor kinase 5 (GRK 5) phosphorylates residues in the C terminus of the β_2-AR, impairing binding of the receptor to NHERF-1 (23). Said in another way, NHERF-1 readily engages

the unphosphorylated receptor, but not β_2-AR phosphorylated by GRK5. This observation provided a critical insight into the dynamic association of the processes that direct the β_2-AR receptor to recycling or degradatory pathways.

The Effect of the β_2-AR/NHERF-1 Interaction on NHE3 and CFTR Activity

Prior studies had demonstrated that β_2-AR stimulation in the kidney proximal tubule raised cyclic AMP levels but that, despite the activation of protein kinase A, β_2-AR agonists increased rather than decreased sodium and water transport (26, 27). Following the characterization of the β_2-AR/NHERF-1 interactions, additional studies were performed to attempt to address this paradox. When renal brush border membrane proteins containing NHE3 were reconstituted into soybean lipid vesicles, the addition of a polypeptide representing C-terminal β_2-AR residues blocked the inhibitory effect of protein kinase A on Na^+-H^+ exchange activity (15). In studies utilizing a fibroblast cell line expressing rat NHE3 and wild-type β_2-AR, treatment with a β_2-adrenergic agonist resulted in stimulation of NHE3 activity, whereas forskolin (which increased cAMP) inhibited the transporter. By contrast, coexpression of a mutated form of β_2-AR that was incapable of binding NHERF-1 resulted in inhibition of NHE3 activity in response to both the β_2-adrenergic agonist and forskolin (15). These experiments were interpreted to indicate that the presence of β_2-AR displaced NHERF-1 binding to NHE3. It is postulated that the NHERF-1 protein cannot accommodate simultaneous occupancy by both β_2-AR and NHE3, and that the agonist-promoted binding of NHERF-1 to β_2-AR represents a mechanism by which β_2-AR stimulation can modulate NHE3 activity.

Following the elucidation of the association between β_2-AR and NHERF-1, three independent groups simultaneously reported binding of the cystic fibrosis transmembrane conductance regulator (CFTR) to NHERF-1 (21, 28, 29). The CFTR C terminus ends in D-T-R-L, a sequence nearly identical to the NHERF-1 binding motif found at the β_2-AR C terminus. Mutation of this motif in the CFTR polypeptide abrogated its association with NHERF-1 (21, 28, 29). Recent studies have indicated that β_2-AR, CFTR, and NHERF-1 form a triple protein complex in airway epithelial cells and that the formation of this complex plays a key role in β_2-AR-mediated regulation of CFTR function (30, 31). These findings suggest another physiologically relevant role for the association between the β_2-AR and the NHERF proteins.

NHERF INTERACTIONS WITH THE κ-OPIOID RECEPTOR

NHERF Binds to the κ-Opioid Receptor

Given the C-terminal amino acid sequences of receptors known to bind to PDZ domain–containing proteins, studies were undertaken to determine whether the

κ-opioid receptor, which has a C-terminal valine, interacts with NHERF-1 (18, 32). It was initially demonstrated, using CHO cells stably expressing the human κ-opioid receptor (hKOR), that NHERF-1 co-immunoprecipitated with hKOR. When the cells were treated with the selective κ-opioid agonist U50,488H, there was a significant increase in the amount of co-immunoprecipitated NHERF-1, indicating that the agonist-occupied receptor had greater binding affinity for NHERF-1 than did the unbound receptor. A polypeptide representing the PDZ-I domain of NHERF-1 (amino acids 1–151) co-immunoprecipitated with hKOR, but a fragment representing PDZ-II[152–358] did not. Thus, like β_2-AR and PTH1R, hKOR preferentially binds to PDZ-I of NHERF-1. Mutant hKOR containing an additional C-terminal alanine (hKOR-A) or glutamic acid residues (hKOR-EE) did not co-immunoprecipitate with NHERF-1, suggesting that the interaction between hKOR and NHERF-1 involved a PDZ domain interaction. Additional studies indicated that purified glutathione-*S*-transferase (GST)-hKOR C-terminal domain (hKOR 334–380) interacted with purified NHERF-1, whereas GST or GST-C terminus of the μ- or δ-opioid receptors did not (32). The C terminus of hKOR-bound NHERF-1 in extracts of CHO cells transfected with the construct. The hKOR C terminus also associated with the PDZ-I but not the PDZ-II domain of NHERF-1.

Although the C-terminal sequence of hKOR, N-K-P-V, is not a canonical PDZ domain–binding motif, these results suggest a PDZ domain interaction between hKOR and NHERF-1. There are, however, differences between β_2-AR and hKOR in their interactions with NHERF-1. Substitution of alanine for serine at the –2 position of β_2-AR or mutation of the C-terminal leucine to valine reduced the interaction with NHERF-1, indicating a class I PDZ interaction (15, 21, 33). Because the –2 position of hKOR is lysine and the C-terminal residue of hKOR is valine, the interaction of hKOR with NHERF-1 appears not to represent a prototypic class I interaction. This suggestion is supported by pull-down studies that indicate that the binding of NHERF-1 to the C terminus of hKOR is much weaker than to the C terminus of β_2-AR (32). Recently, Heydorn et al. (34) screened the C termini of 59 GPCRs for interaction with NHERF-1 and found that β_2-AR was the only one that yielded a positive result, whereas hKOR did not interact with NHERF-1 in this assay. As these investigators used relative signals and β_2-AR yielded a very strong signal, weaker interactions may have been overlooked. In pull-down assays, the hKOR C terminus–bound NHERF-1 expressed in CHO cells or endogenous NHERF-1 in the brain with apparently higher affinities than did purified NHERF-1 (P. Huang & L.-Y. Liu-Chen, unpublished observations). Such findings may suggest that there are cell-specific posttranslational modifications of NHERF-1 or that accessory proteins affect the binding characteristics. Whatever the explanation, the results with hKOR suggest that NHERF-1, and particularly PDZ-I, has broad binding specificity by virtue of the conformation of the binding groove when associated with different PDZ-I targets. It is, in fact, the flexibility and diversity of PDZ- I of NHERF-1 that may allow interaction with a subset of GPCRs.

NHERF-1 Affects the Trafficking of the κ-Opioid Receptor

Treatment of CHO cells expressing hKOR with U50,488H resulted in a 30% reduction in the number of hKOR receptors (18, 35). Of interest, expression of NHERF-1 in CHO cells coexpressing hKOR abolished U50,488H-induced downregulation of the receptor (18). By contrast, U50,488H-induced downregulation of the mutant receptors hKOR-A and hKOR-EE, which do not co-immunoprecipitate with NHERF-1, was not affected by expression of NHERF-1. Other studies have indicated that interactions between NHERF and the ERM proteins are important in the regulation of hKOR (36). Expression of NHERF-1 (1-298) containing the PDZ domains but lacking the ERM-binding domain did not block downregulation of hKOR, indicating that binding of NHERF-1 to the cortical actin cytoskeleton is critical for its inhibitory effect on downregulation. As KOR internalization is required for downregulation, a reduction in downregulation may be due to a decrease in KOR internalization (35). Alternatively, because internalized receptors either are routed for degradation in lysosomes and/or proteasomes or recycled back to plasma membranes, attenuation in downregulation may be attributed to enhanced recycling. Additional studies have now indicated that coexpression of NHERF-1 accelerated the recycling of internalized hKOR without affecting U50,488H-induced internalization (18). By contrast, expression of NHERF-1 did not affect U50,488H binding affinity, U50,488H-stimulated [^{35}S]GTPγS binding, activation of p42/p44 MAP kinase, or U50,488H-induced desensitization of hKOR. These results indicate that NHERF-1 binds to the C terminus of the hKOR; this association appears to serve as a signal for internalized hKOR to be sorted to the recycling pathway.

Physiologic Consequences of NHERF-1/κ-Opioid Receptor Association

The physiologic consequences of NHERF-1 interaction with the κ-opioid receptor were determined by measuring Na^+-H^+ exchange activity in OK proximal tubule epithelial cells. OK cells demonstrate the presence of κ-opioid binding sites and, using pull-down techniques, the hKOR C terminus interacted directly with endogenous OK NHERF-1 (18, 37). Incubation of OK cells with U50,488H significantly enhanced Na^+-H^+ exchange (32). U50,488H-stimulated Na^+-H^+ exchange was blocked by naloxone but not by pertussis toxin pretreatment, indicating that it is mediated by KOR but independent of G_i/G_o proteins. In OKH cells, a subclone of OK cells expressing a much lower level of NHERF-1, U50,488H had no effect on Na^+-H^+ exchange. By contrast, in OKH cells, as in OK cells, U50,488H stimulated p44/p42 MAP kinase phosphorylation via κ-opioid receptors and pertussis toxin-sensitive G proteins. Stable transfection of NHERF-1 into OKH cells restored the stimulatory effect of U50,488H on Na^+-H^+ exchange. Thus, NHERF-1 binds directly to KOR, and this association plays an important role in accelerating Na^+-H^+ exchange. How the κ-opioid receptor interacts with NHERF-1

to stimulate NHE3 in OK cells is not currently well understood. The hKOR C terminus facilitates oligomerization of NHERF-1, similar to the C-terminal domain of the platelet-derived growth factor receptor (PDGFR) and β_2-AR (13, 19, 32). NHERF-1 oligomers may represent a pool of inactive NHERF-1 that is incapable of inhibiting NHE3 (14, 38). A working hypothesis is that there is a basal level of PKA activity in OK cells that mediates NHERF-1-associated inhibition of NHE3. Activation of the KOR enhances its association with and oligomerization of NHERF-1. This, in turn, eliminates the inhibition of NHE3 by NHERF-1, unmasking the observed stimulatory effect.

NHERF INTERACTIONS WITH THE PARATHYROID HORMONE RECEPTOR

Structural Determinants of PTH1R Binding to NHERF

Parathyroid hormone (PTH) receptors transduce the effects of PTH and of PTH-related peptide. The type 1 PTH receptor (PTH1R) is a Class II GPCR and is widely expressed in tissues responsible for mineral ion homeostasis. PTH1R stimulates adenylyl cyclase and/or phospholipase C (PLC), and it has been established that multiple G proteins mediate the bifurcating signaling pathways activated by PTH (39, 40). The PTH1R binds to PDZ-I of NHERF-1, but to PDZ-II of NHERF-2 (41). The C-terminal amino acid sequence of PTH1R, E-T-V-M, is consistent with a class 1 PDZ-binding domain motif. Mutation of E, T, or M of the PTH1R PDZ recognition domain prevents binding to full-length NHERF-1 or NHERF-2 (16, 17). The -1 position is permissive, and mutation to alanine does not interfere with the interaction with NHERF-2. Mahon and Segre (16) identified an 18-amino acid C-terminal PTH1R fragment (residues 573–591) that interacted with NHERF-1 in pull-down assays. A smaller receptor fragment, residues 583–591, however, did not, implying that the interaction between NHERF-1 and the PTH1R extends upstream to include some residues of the C-terminal 18 amino acids. The residues in this region were identified as E585 and E586. This finding suggests that these acidic residues contribute to stabilizing the interaction of the PTH1R with NHERF-1. From studies using a truncated form of the PTH1R lacking virtually all of the C-terminal intracellular tail, Sneddon et al. (42) found that although interaction of PTH1R, through its PDZ-binding domain with NHERF-1, modified the response to activating and nonactivating ligands, it was not required for receptor internalization. NHERF-1 lacking the ERM domain was incapable of suppressing PTH1R endocytosis evoked by PTH[7–34], a PTH fragment missing the N terminus required for adenylyl cyclase activation. Preliminary experiments have indicated that mutations in the PDZ-I-binding groove reduced the effect of NHERF-1 on PTH[7–34]-induced internalization (41). Actin colocalizes with NHERF-1 and the PTH1R in apical domains of OK proximal tubule–like opossum kidney cells (29). In OKH cells expressing little or no NHERF-1, PTH1Rs were diffusely

distributed throughout the cytoplasm with some punctate localization in apical cell membranes and along the basolateral membrane. Treatment of the cells with cytochalasin D, a membrane-permeant inhibitor of actin polymerization, promoted actin aggregation and disrupted apical membrane PTH1R localization, attended by redistribution to the cytoplasm. Colchicine, a microtubule inhibitor, had no effect on PTH1R endocytosis (17, 43). In contrast to cells expressing NHERF-1, cytochalasin D had no effect on PTH1R endocytosis in response to PTH[1–34] in cells deficient in NHERF-1. When considered together, these findings suggest that the interaction between NHERF-1 and cortical actin plays a role in internalization of PTH1R and/or stabilizing PTH1R in the plasma membrane.

NHERF and the Trafficking of PTH1R

PTH1R, like other G protein–coupled receptors, undergoes cyclical receptor activation, desensitization, and internalization. Following endocytosis, PTH1R either can be recycled to the cell membrane, resulting in receptor resensitization, or targeted for degradation, leading to receptor downregulation (41, 42, 44). In distal tubule cells lacking endogenous NHERF-1, PTH[1–31], a PTH fragment that lacks the 32–34 sequence required for PTH1R activation of protein kinase C, had no discernible effect on PTH1R internalization, despite the fact that it stimulated adenylyl cyclase and phospholipase C (PLC) (44). PTH[1–34], a polypeptide that recapitulates all the classic effects of full-length PTH[1–84], promoted receptor endocytosis. PTH[7–34] lacking the N terminus required for adenylyl cyclase activation induced prompt PTH1R internalization that was faster and greater in magnitude than that observed with PTH[1–34]. Similar results were obtained with PTH[1–84] and PTH[7–84] (17). These observations indicate a striking dissociation between receptor activation and inactivation by native peptides. By contrast, the profile of responses was markedly different in proximal tubule cells that constitutively express NHERF-1. In proximal tubule cells, PTH[7–34] and PTH[1–31] had little or no effect on PTH1R endocytosis, whereas PTH[1–34] caused prompt internalization. Distal tubule cells and rat osteoblast-like cells, which also display little constitutive NHERF-1 expression, responded to PTH[7–34] and PTH[1–34] in a manner similar to proximal tubule cells when NHERF-1 was stably expressed. Conversely, proximal tubule cells transfected with a dominant negative form of NHERF-1 assumed a distal tubule response profile when treated with PTH[7–34]. These results are consistent with the view that NHERF-1 conditionally modulates the response to PTH fragments that are full agonists or antagonists. The ability of peptides with only weak affinity for the PTH1R to promote conformational changes is enhanced in the absence of NHERF-1. This suggests that NHERFs stabilize the receptor in the membrane such that only full agonists are able to elicit biological responses. These observations also suggest that PTH1R activation and endocytosis are mediated through distinct structural states that depend on the nature of the specific interactions between ligand and receptor.

NHERF Binding to PTH1R Functions as a Molecular Switch in the Generation of Downstream Signals

When expressed in PS120 fibroblasts that contain little or no NHERF-1 or NHERF-2, PTH1R signaled robustly and exclusively through adenylyl cyclase (16). When NHERF-2 was coexpressed, cAMP formation was decreased, whereas PLC signaling and inositol phosphate formation were dramatically increased. These important and novel findings suggest a unique role for NHERF-2 as a bimolecular signaling switch. Pertussis toxin treatment of PS120 cells coexpressing PTH1R and NHERF-2 inhibited PTH activation of PLC and partially restored activation of adenylyl cyclase. This indicates that PTH stimulates G_i/G_o proteins when the PTH1R is bound to NHERF-2. These findings are consistent with the idea that, upon activation of G_i/G_o, dissociation of the $\beta\gamma$- subunits activates PLC-β, whereas the α_1-subunits inhibit adenylyl cyclase (40).

NHERF, PTH1R Signaling, and Sodium-Dependent Phosphate Transport

By contrast to wild-type OK cells, PTH stimulated adenylyl cyclase but had no effect on phosphate transport in OKH cells (45). When OKH cells were transfected with NHERF-1, inhibition of phosphate transport by PTH was restored. Although introducing NHERF-1 in OKH cells reduced cAMP signaling and promoted inositol phosphate formation, there was little correlation between the changes in second-messenger formation and phosphate transport. Moreover, inhibiting PKA or PKC pathways failed to curtail PTH-sensitive phosphate transport. Thus, the precise mechanism by which NHERF-1 transduces PTH signals to inhibit phosphate transport in OK cells remains unresolved.

NHERF INTERACTIONS WITH THE RECEPTOR TYROSINE KINASES

In addition to associating with G protein–coupled receptors, the NHERF proteins have been found to interact with various receptor tyrosine kinases. These kinases include the platelet-derived growth factor receptor (PDGFR) and epidermal growth factor receptor (EGFR).

NHERF Binds to the Platelet-Derived Growth Factor Receptor

The possibility of an association between NHERF-1 and PDGFR was first examined because of the striking similarity between the C termini of PDGFR (D-S-F-L) and β_2-AR (D-S-L-L). NHERF-1 was found to associate with PDGFR and potentiate the receptor's signaling activity (19). It had been established previously that minimal truncations to the distal C terminus of PDGFR strongly impaired

PDGFR signaling, but the relevant protein-protein interactions disrupted by such truncations were completely unknown (46). Characterization of the effects of the association between PDGFR and NHERF on the receptor's activity provided a specific molecular mechanism to help explain the importance of the distal PDGFR C terminus in controlling the receptor's signaling.

The interaction between PDGFR and NHERF is of special importance in mediating PDGFR reorganization of the actin cytoskeleton (47). Fibroblasts expressing mutant PDGFRs incapable of binding NHERF exhibit markedly reduced migration as well as alterations to their actin cytoskeleton (48). Recent studies have indicated that the interaction between PDGFR and NHERF-1 can be disrupted by phosphorylation of PDGFR by GRK2. GRK2 phosphorylates the serine residue at the –2 position on the PDGFR C terminus and, by blocking binding to NHERF proteins, alters PDGFR activity (49). These findings parallel earlier experiments that demonstrated that the β_2-AR/NHERF interaction can be disrupted by phosphorylation of the β_2-AR C terminus by GRK5 (23). Taken together, these studies suggest that GRK-mediated phosphorylation of receptor C termini may be a general mechanism by which interactions with the NHERF proteins are regulated.

NHERF Binds the C Terminus of the Epidermal Growth Factor Receptor

Despite the fact that the C terminus of the epidermal growth factor receptor (EGFR) does not terminate in a canonical NHERF PDZ-binding motif, EGFR does associate with NHERF-1. A yeast two-hybrid screen of a mouse embryo cDNA library for potential EGFR-interacting proteins, using the distal EGFR C terminus as bait, revealed four positive clones, all of which corresponded to NHERF-1 (20). The interaction between EGFR and NHERF-1 is likely mediated by an internal peptide motif within the EGFR C terminus. The association between EGFR and NHERF-1 appears to stabilize EGFR at the cell surface and prevent its agonist-induced receptor downregulation, which parallels the aforementioned effects of NHERF-1 in preventing agonist-induced downregulation of β_2-AR (20, 23).

CONCLUSIONS

Study of the association between specific hormone and growth factor receptors and the two NHERF isoforms, NHERF-1 and NHERF-2, has indicated a role for these adaptor proteins beyond the regulation of ion transporters and channels. In fact, receptors currently represent the largest group of NHERF targets identified and suggest a critical role of these adaptor proteins in signal transduction. Emerging evidence suggests that NHERFs use a variety of modes to elicit hormonal control of physiological processes, ranging from the assembly of signaling complexes containing protein kinase A and its substrates, such as NHE3 and CFTR; to the trafficking and tethering of membrane proteins to promote signaling, as

discussed for PDGFR; to the modulation of second-messenger generation, as seen for PTH1R. Recent studies suggest additional targets of NHERF-1 such as Gαq and coatomer proteins may also contribute to hormone signaling as well as receptor internalization and recycling (50, 51).

As study of the role of the NHERF proteins in regulating components of intracellular signaling pathways and other cell machinery expands, the remarkable diversity of these PDZ adaptor proteins is emphasized. Studies of EGFR and other targets whose C termini bind one or both NHERF PDZ domains suggest that the structural determinants that mediate their association with NHERF may be quite different from those of other targets studied to date. This may further expand the array of NHERF targets present in mammalian tissues. Even among those proteins that bind NHERF via their C termini, such as β_2-AR and hKOR, there appear to be significant differences in their affinities for the NHERF PDZ domains. This suggests that in diseases, such as aggressive forms of human breast cancer, that have been associated with elevated NHERF-1 expression, the disease state may arise from the increased association of NHERF-1 with a wide range of cellular targets. Mutations in some NHERF targets, such as the ERM protein merlin (also known as NF2) or transporters such as CFTR and Npt2a, have been linked with their altered association with NHERF, and it has been suggested that disruption of cellular NHERF complexes may contribute to human disease. Finally, recent studies have identified mutations in the NHERF proteins in specific cancers and in individuals with renal disease. These studies also suggest that defects in NHERF-regulated hormone signaling and other cellular functions may be critical in the development of these diseases.

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