



Role of Nck in T Cell Receptor Activation

Ichaya Yiemwattana^{1*}, Jatuporn Ngoenkam² and Sutatip Pongcharoen³

¹Department of Preventive Dentistry, Faculty of Dentistry

²Department of Microbiology and Parasitology, Faculty of Medical Science

³Department of Medicine, Faculty of Medicine, Naresuan University, Phitsanulok, Thailand

* Corresponding author. E-mail address: ichayak@yahoo.com

Summary

The non-catalytic region of tyrosine kinase (Nck) is an important regulator of the cytoskeletal reorganization and of the formation of the immune synapse between T cells and antigen presenting cells (APCs). Nck acts as a link between the TCR:CD3 complex and signaling molecules that mediate cytoskeleton rearrangement and T cell activation. The intracellular T cell signaling both in an immunoreceptor tyrosine-based activation motif (ITAM)-requiring mechanism and in a non-ITAM-requiring mechanism may be regulated by Nck. Therefore, this review attempts to summarise the critical role of Nck in T cell receptor activation. The subsequent applications may contribute to the usage of Nck as a target for pharmacological modulation of the immunopathologic conditions.

Keywords:Nck, adaptor protein, TCR activation, T cells

Introduction

T cells are the key cellular constituents of the immune system that possess the capacity to directly destroy cells infected with a pathogen and have a central role in stimulating and coordinating with other components of the immune response, such as B cells. The TCR:CD3 surface expression is essential for T cell development and function and is therefore a key element in the initiation of the adaptive immune response (Dave, et al., 1997; Haks, Krimpenfort, Borst, & Kruisbeek, 1998; Love, et al., 1993; Wang, et al., 1999). The TCR:CD3 complex recognizes a foreign peptide presented by a major histocompatibility (MHC) molecule. Binding of TCR:CD3 complex to peptide-MHC complex molecule initiates a specific immune response. T cell signaling is transduced not by the TCR itself but by invariant proteins called CD3 and $\zeta\zeta$ within the TCR complex. The ITAMs, which are located in the cytoplasmic domains of CD3 and ζ chains are

phosphorylated. This results in activation of downstream signaling molecules and initiation of signaling complexes (Lin, & Weiss, 2001; Rudolph, & Wilson, 2002; Werlen, & Palmer, 2002).

Numerous proteins and protein complexes constitute the T cell signaling pathway. Signaling pathways usually start with the alteration of intracellular molecules of receptor proteins that create various signal transduction cascades. The signaling molecules, which regulate the formation of multimeric protein complexes, can be divided into three groups: (1) enzymes, such as Src family tyrosine kinases, phospholipase C γ and RasGTPase-activating protein (RasGAP), (2) regulator molecules, such as the Vav family of Rho guanine nucleotide exchange proteins, Cbl and signal transducer and activator of transcription (STAT) proteins, and (3) adaptor proteins without any known enzymatic activities (Buday, Wunderlich, & Tamas, 2002). Adaptor proteins usually contain several domains within their structures (e.g., Src homology 2



(SH2) and SH3 domains), allowing specific interactions with several other specific proteins. These proteins mediate specific protein-protein interactions that drive the formation of protein complexes for the activation of signal transduction cascades inside the cell. This review will focus on a role of Nck that is important for T cell activation and emphasize the various ways in which this adaptor carries out its essential functions.

The Nck Family of Adaptor Proteins

Nck (non-catalytic region of tyrosine kinase) is a cytosolic adaptor molecule of 47-kDa. Nck is expressed in a wide variety of cells and tissues. The human Nck cDNA was originally cloned by Lehmann, Riethmuller, and Johnson (Lehmann, Riethmuller, & Johnson, 1990). Using monoclonal antibodies recognizing the melanoma specific MUC18 antigen, Nck was identified as a false positive during the screening of a melanoma cDNA expression library. Nck has been cloned for the second time by virtue of its ability to bind the phosphorylated C-terminal tail of the epidermal growth factor (EGF) receptor in the cloning of receptor targets (CORT) screening technique (Skolnik, et al., 1991). The murine homolog of Nck, termed Grb4 was later cloned in an attempt to identify novel SH2 domain containing

proteins (Margolis, et al., 1992). In human cells, the Nck family comprises two highly related members (Nck1/Nck α and Nck2/Nck β , also termed Grb4). Nck1 and Nck2 display 68% identity of overall amino acid residues. Notably, the largest differences are mainly located in the linker regions between the interaction modules (Figure 1). While the human nck-1 gene has been localized to the 3q21 locus of chromosome 3, the nck-2 gene can be found on chromosome 2 at the 2q12 locus (Huebner, et al., 1994; Vorobieva, et al., 1995). However, hardly any Nck1- or Nck2-specific downstream target has been identified so far. In fact, in many instances the interactions have not been clearly attributed to Nck1 or Nck2. Therefore, Nck1 and Nck2 are generally termed Nck. Nck is a prototype of SH domain-containing adaptor proteins that facilitate multiple interactions with other related proteins and form functional signaling complexes. Nck molecule consists of a single C-terminal SH2 domain, which binds to phosphotyrosine residues, and three N-terminal SH3 domains, which bind to proline-rich sequence (PRS) within specific peptide sequence contexts of proteins. Nck has been reported to be able to interact with approximately 60 different proteins, suggesting that it is used by a wide variety of intracellular signaling pathways (Buday, et al., 2002).

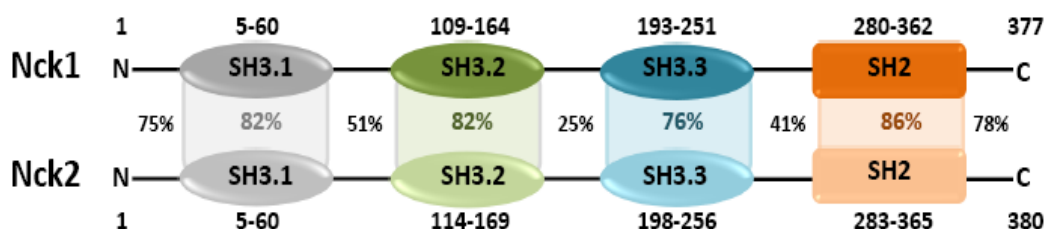


Figure 1 Diagram illustrating the architecture of Nck family



Figure 1 Diagram illustrating the architecture of Nck family. (A) Nck1 and Nck2 contain identical domains, which are three Src Homology 3 (SH3) domains at N-terminus and a Src Homology 2 (SH2) domain at C-terminus. The percent similarity of amino acids of the domains and linker regions between Nck1 and Nck2 are shown. The numbers of amino acid length of Nck1 and Nck2 are shown at the C-terminus.

Nck can bind to a number of phosphotyrosine proteins, such as activated EGF and platelet-derived growth factor (PDGF) receptor tyrosine kinases (Chou, Fajardo, & Hanafusa, 1992; Li, Hu, Skolnik, Ullrich, & Schlessinger, 1992; Meisenhelder, & Hunter, 1992), downstream of tyrosine kinases (Dok) (Noguchi, et al., 1999), and SH2-domain-containing leukocyte protein of 76 kDa (SLP-76) (Wunderlich, Farago, Downward, & Buday, 1999) via its SH2 domain. SH2 domain links the Nck proteins to upstream tyrosine-phosphorylated receptor and cytosolic proteins to downstream signaling pathways. To date, there are more than twenty tyrosine-phosphorylated proteins that can interact with SH2 domains of Nck1 and Nck2 (Lettau, Pieper, & Janssen, 2009). Although SH2 domains of both Nck isoforms can recognize the consensus motif of pYDxV (AYST) x (DEC) (when x is any amino acid) on the partner proteins, several pieces of evidence have indicated some non-overlapping functions of SH2 domains between Nck1 and Nck2 (Chen, She, Kim, Woodley, & Li, 2000; Lettau, et al., 2009; Nishimura, et al., 1993; Pramatarova, et al., 2003). Upon TCR triggering, SH2 domain of Nck is recruited to phosphorylated SLP-76 via two tyrosine-phosphorylated sites, Tyr113 (YESP) and Tyr128 (YESP) (Wunderlich, et al., 1999).

SH3 domains of Nck mediate the interaction with their downstream target proteins, which contain PRS (Lettau, et al., 2009). Early biochemical studies have identified several proteins that can interact with one or more of the Nck SH3 domains. Those proteins

are, for examples, son of sevenless (Sos) (Hu, Milfay, & Williams, 1995; Okada, & Pessin, 1996), Vav (Ramos-Morales, Druker, & Fischer, 1994), Wiskott-Aldrich syndrome protein (WASP) (Rivero-Lezcano, Marcilla, Sameshima, & Robbins, 1995), Nck-associated p21-activated kinase 1 (PAK1) (Bokoch, et al., 1996) and CD3E (Gil, Schamel, Montoya, Sanchez-Madrid, & Alarcon, 2002). To date, over thirty proteins have been reported to interact with SH3 domains of Nck1 and Nck2. Moreover, several Nck ligands bind to more than one SH3 domain of Nck. This suggests that a cooperative interaction is necessary for tight complex formation (Wunderlich, Goher, Farago, Downward, & Buday, 1999). Nck utilizes the specificity of its individual SH3 domains to facilitate multiple interactions with different associated proteins.

Nck can recruit proline-rich proteins to the plasma membrane. The proline-rich proteins are also recruited by Nck to the multiple protein complexes found either in the cytoplasm or in association with the actin cytoskeleton. It has been reported that Nck is involved in many aspects of tissue development, immune cell activation and tumorigenesis. These processes, which depend on changes in cell polarity, morphology and migration, are regulated by protein complexes formed by Nck (Buday, et al., 2002; Lettau, et al., 2009). It has been reported that Nck acts as a link between extracellular and intracellular signaling molecules as well as cytoskeleton (Lehmann, et al., 1990; Park, 1997; Ullrich, & Schlessinger, 1990). In T cells, Nck plays a pivotal role in the TCR-induced reorganization of the actin cytoskeleton and the formation of the immunological synapse between T cells and antigen presenting cells (APCs). Two different models for the association of Nck with TCR/CD3 complex have been proposed. In an ITAM-requiring pathway, Nck is recruited to the TCR complex via phosphorylated SLP-76



(Wunderlich, et al., 1999), another central constituent of the membrane proximal activation complex. In a non-ITAM-requiring pathway, dependent on an activation-induced conformational change in the CD3 ϵ subunits, a direct binding of Nck to components of the TCR/CD3 complex has been reported (Gil, et al., 2002). In this regard, activation-dependent association between Nck and CD3 ϵ PRS occurs prior to ITAM phosphorylation.

Besides Nck, there are a number of SH2/SH3 adaptor proteins that link upstream signal from TCR, through their SH2 domains to different downstream signaling pathways, through their SH3 domains. These proteins are Crk, Grb2 (growth factor receptor-bound protein 2), Gads (Grb2-related adaptor downstream of Shc), and p85. Their functions in T cell activation are discussed briefly. Crk (Crk-II and Crk-L) consists of a single SH2 domain and two SH3 domains located at N- to C-terminus, respectively, in order SH2-SH3-SH3 (Lin, & Weiss, 2014). Crk is associated with phosphorylated Cbl via its SH2 domain upon TCR triggering, which is required for TCR-regulated integrin adhesion (Nolz, et al., 2008). Grb2 is built of an SH2 domain and two SH3 domains, in order SH3-SH2-SH3. The Grb2 SH3 domain constitutively interacts with SOS and also upon TCR stimulation. This Grb2/SOS interaction is required for TCR-induced Ras-Erk signaling cascade (Smith-Garvin, Koretzky, & Jordan, 2009). Gads is an SH3-SH2-SH3 adaptor protein implicated in the linking of phosphorylated LAT, through Gads SH2 domain, to SLP76, through Gads PRS upon TCR stimulation. The formation of this complex is essential for the activation of various signaling pathways such as MAPK and Ca²⁺ pathways (Yoder, et al., 2001). The p85 is the SH3-SH2-SH2 adaptor protein, which is a regulatory subunit of PI3K (phosphatidylinositol 3-kinase) (McCarty,

1998). Following TCR engagement, the p85 SH2 domain mediates the localization of PI3K to plasma membrane, where PI3K is activated and controls the transcription of cytokine genes (Kang, Schneider, & Rudd, 2002).

Thus, it is possible that the recruitment of Nck to CD3 ϵ interferes with subsequent phosphorylation of the CD3 ϵ ITAM by Src kinases. To date, the function of Nck in T cell receptor signaling has not been completely understood.

Nck and T Cell Effector Function

Actin Reorganization in T Cell Activation

Actin cytoskeleton consists of a network of filaments, which is composed of individual actin protein. There are two forms of actin protein in cells, a monomeric form (globular or G-actin) and a polymerized form (filamentous or F-actin) (Blasutig, 2008). TCR activation triggers actin dynamics for controlling vital T cell responses, such as T cell adhesion, motility, and proliferation. These depend on the recruitment of Nck and WASP to the site of TCR activation. Upon optimal TCR engagement, ITAMs are phosphorylated, resulting in activation of zeta-chain (TCR) associated protein kinase 70 kDa (Zap70). Activated Zap70 then phosphorylates linker for activation of T cells (LAT) and SLP-76. The phosphorylated SLP-76 is the site for recruitment of SH2 containing proteins. These are Nck, Vav1, and the nucleation-promoting factors (NPFs) including the WASP, WASP-family verprolin-homologous protein-2 (WAVE2) and hematopoietic cell lineage-specific protein 1 (HS1) (Wu, & Koretzky, 2004).

Since Nck has no intrinsic ability to mediate actin polymerization, Nck acts as a scaffold protein to recruit other signaling proteins to form signaling complexes that can induce actin polymerization. Nck

can interact with phosphorylated SLP-76 through its SH2 domain and can associate with PAK, Vav1, and WASP (through its SH3 domains Bubeck, et al., 1998; Bunnell, et al., 2002; Fischer, et al., 1998; Galisteo, Chernoff, Su, Skolnik, & Schlessinger, 1996; Rivero-Lezcano, et al., 1995; Wunderlich, et al., 1999).

The main function of the multidomain adapter protein WASP is the activation of the actin-related proteins 2 and 3 (Arp2/3) complex. Arp2/3 is the seven-subunit complex that is in an inactive conformation in resting state, but can be activated by

WASP and WAVE2 upon T cell stimulation. The recruitment of Arp2/3 to WASP coupled with actin monomer induces the formation of branched actin filament networks (Figure 2) (Panchal, Kaiser, Torres, Pollard, & Rosen, 2003). Nck not only passively interacts with WASP and may thus recruit WASP to molecular activation clusters (Rivero-Lezcano, et al., 1995), but also modulates WASP activity (Rohatgi, Nollau, Ho, Kirschner, & Mayer, 2001). Therefore, this adapter protein also regulates activation-dependent reorganization of TCR-associated signaling complexes and platforms.

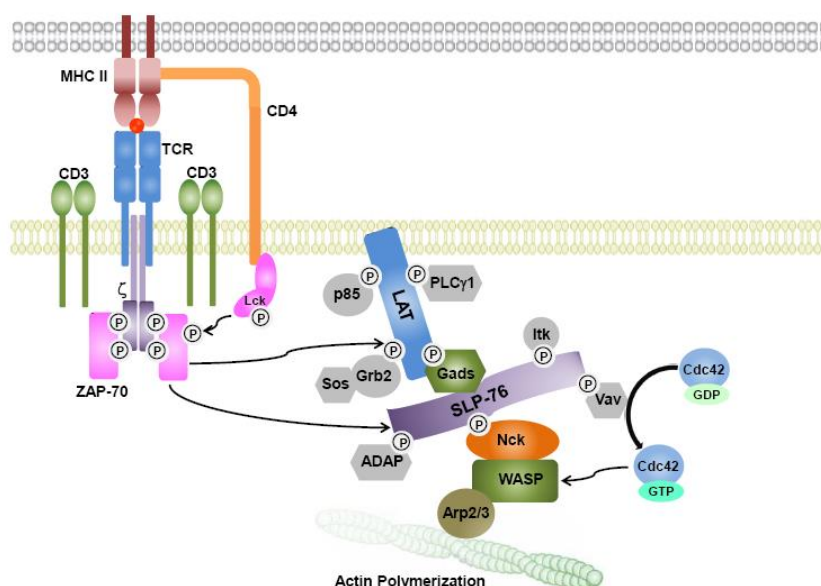


Figure 2 Schematic diagram of molecular signaling cascades that link the TCR signaling to cytoskeleton rearrangement

Figure 2 Schematic diagram of molecular signaling cascades that link the TCR signaling to cytoskeleton rearrangement. After TCR ligation with its ligand, antigenic peptide bound on the MHC molecule that is expressed on the antigen presenting cells (APCs) surfaces. This leads to the phosphorylation of ITAMs located at the cytoplasmic tails of CD3 and ζ molecules by protein tyrosine kinase Lck that is associated with the cytoplasmic tail of CD4 molecule. ZAP-70 is then recruited to the

phosphorylated ITAMs, where it is activated. The activated ZAP-70 in turn phosphorylates LAT and SLP-76. The phosphorylated LAT serves as docking sites for adaptor proteins such as Grb2 and Gads. Subsequently, SLP-76 is recruited to LAT-nucleated complex via Gads. Like LAT, SLP-76 is an adaptor protein that contains the sites for interaction with various proteins such as Nck, Vav, Itk and ADAP. Nck then recruits the adaptor protein WASP. The GEF Vav promotes the exchange of Cdc42-GDP to



Cdc42-GTP that is essential for WASP activation. WASP activates the Arp2/3 complex, which then induces the formation of branched actin filament networks.

Nck and signaling within T cells

Many models have been proposed for how TCR signaling is initiated, including TCR complex conformational change. Conformational change in the TCR complex upon antibody binding has been proposed to explain studies of T cell signaling where receptor multimerization or the avidity of antibodies for the receptor do not sufficiently explain the biochemical results (Janeway, 1995). This idea is attractive because the ITAMs in the cytoplasmic domains of these components are the proximal targets of tyrosine phosphorylation. Tyrosine residues within these motifs, once phosphorylated by the Src family tyrosine kinase (SFK), become binding sites for different SH2-containing proteins, including the tyrosine kinase ZAP70 and then initiate a cascade of downstream signaling events that result in nuclear transcriptional changes as well as cellular morphological changes (Lin, & Weiss, 2001). Several studies have indicated that activated Zap70 promotes recruitment and phosphorylation of downstream signaling molecules including SLP-76, Vav1, a Rho family exchange factor, and Nck (Alberola-Ila, Takaki, Kerner, & Perlmutter, 1997; Bubeck, et al., 1998; Galisteo, et al., 1996; Lin, & Weiss, 2001). The assembly of this multiprotein complex regulates target effectors that interact through Nck, including PAK1. Consistent with this model, TCR-dependent activation of PAK1 and inducible association of PAK1 with Nck have been reported. PAK1 is required for the activation of nuclear factor of activated T-cells (NFAT). Prevention of Nck to interact specifically with PAK1 by the expression of a dominant negative allele of

Nck containing a W143K mutation at the second SH3 domain inhibits TCR-mediated NFAT activation. Moreover, PAK1 activation is not necessary for TCR-dependent c-Jun N-terminal kinase (JNK) activation, one component of transcription factor activating protein-1 (AP-1) activation pathway. These results indicate that Nck-PAK1 interaction is required for TCR-mediated NFAT activation, but not for JNK activation (Yablonski, Kane, Qian, & Weiss, 1998). Another current model for TCR signaling is a non-ITAM-requiring mechanism. In this regard, CD3 ϵ cytoplasmic tail undergoes a conformational change that exposes the PRS for Nck binding upon multivalent ligands-mediated TCR triggering (Gil, et al., 2002; Gil, Schrum, Alarcon, & Palmer, 2005). This association is mediated by the first SH3 domain of Nck. The exposure of TCR transgenic mouse T cells to antigen-pulsed presenting cells has been shown to promote Nck binding to TCR/CD3 complex. Nevertheless, an Nck-CD3 ϵ interaction-inducing antibody, namely OKT3, can induce both Interleukin-2 (IL-2) release and CD69 expression in Jurkat cells (Gil, et al., 2002). Moreover, Nck recruitment to CD3 ϵ occurs earlier than phosphorylation of the CD3 ϵ ITAM and is independent of tyrosine kinase activity. In addition, disruption of the Nck/CD3 ϵ interaction inhibits immunological synapse maturing and T cell activation. Through a cascade of T cell activation, T cells must efficiently recognize APCs or target cells via several complex cytoskeleton-dependent processes, including integrin-mediated adhesion, immunological-synapse formation, cellular polarization, and receptor signaling. The dynamic actin reorganization is required to coordinate these processes and ultimately controls T-cell recognition and activation. Therefore, the direct recruitment of Nck and associated actin regulatory proteins such as



WASP, WIP or PAK1 to CD3 ϵ of TCR might exhibit an alternative means to a direct link between T cell activation to the cytoskeletal reorganization.

The protease-sensitivity assay has revealed that the cytoplasmic tails of CD3 ϵ subunit adopt a compact structure in the TCR triggering and conformational change is transmitted to the tails of CD3 ϵ subunits. In non-stimulated cells, the CD3 subunits are in a closed conformation, marking the PRS and preventing its interaction with Nck (Risueno, Schamel, & Alarcon, 2008). These would account for the ability of Nck to bind to CD3 ϵ in resting T cells. However, because Nck and some of its binding proteins, including SLP76, are tyrosine phosphorylated (Buday, 1999; Lin, & Weiss, 2001), it is proposed that the conformational change that promotes Nck recruitment and the activation of phosphotyrosine kinases as a consequence of TCR-CD3 cross-linking soon come together into a common activation pathway (Gil, et al., 2005).

A recent study in mice has shown that Nck-defective T cells fail to proliferate and to produce IL-2 upon stimulation with anti-CD3 ϵ antibody but not with the DAG mimetic phorbol 12-myristate 13-acetate (PMA) and ionomycin (Roy, et al., 2010). The unmodified response of Nck-deficient T cells to PMA and ionomycin stimulation is due to signaling pathway that bypasses the TCR signaling apparatus. PMA induces a PKCs-mediated oxidative signal and RasGRP activation, whereas ionomycin induces intracellular calcium mobilization. Nck deletion also impairs TCR-mediated calcium mobilization and ERK phosphorylation in activated T cells. These results suggest the involvement of Nck in proximal TCR signaling. Our recent studies have also shown that Nck1 is important in TCR/CD3-mediated activation involving the extracellular-signal-regulated kinase (Erk) phosphorylation pathway, as evidenced by the inhibition of T cell

function, particularly to an impairment CD69 expression and IL-2 production when its expression is down-regulated (Yiemwattana, et al., 2012). Furthermore, down-regulation of Nck1, but not Nck2, impairs TCR-induced phosphorylation of the mitogen-activated protein kinase kinase (MEK), activation of the AP-1 and NFAT transcription factors and subsequently, IL-2 and CD69 expression (Ngoenkam, et al., 2014). In this scenario, Nck1 could bridge TCR activation to downstream signaling mechanisms and formation of Nck-mediated signaling complex gives rise to the propagation of proximal TCR signals, leading to functional events.

However, conflicting data indicate that interaction between the CD3 ϵ PRS and Nck is not required for T cell development and function (Szymczak et al., 2005). Mice lacking CD3 ϵ PRS motif have normal number and percentage of T cell subsets in the thymus and spleen, with out apparent defect in positive or negative selection. Furthermore, the CD69 expression and proliferative response of mutant T cells to staphylococcal enterotoxin B (SEB) and anti-CD3 antibody is normal. Biochemical and structure studies have demonstrated that the Nck-CD3 ϵ is capable of downregulating T cell activation by inhibiting of CD3 ϵ ITAM phosphorylation and subsequent ITAM-dependent recruitment of downstream signaling molecules by Fyn and Lck kinases in vitro and/or reducing TCR cell surface expression upon physiological stimulation in mouse primary lymph node cells (Takeuchi, et al., 2008). In this context, Nck binds to a noncanonical PxxDY region juxtaposed to the PRS in CD3 ϵ chain. Accordingly, the PxxDy motif encompasses a putative internalization motif, YxxI/L, although this sequence may behave only as a weak internalization signal of TCR/CD3 ϵ endocytosis (Borrito, et al., 1999). Moreover, it has been suggested that the



capacity of CD3 ϵ to bind Nck may serve to amplify TCR signaling and to promote ITAM phosphorylation in response to weak TCR stimuli (Tailor, et al., 2008). In this scenario, strong MHC agonists triggered cytokine release in both naïve and differentiated murine CD8 $^{+}$ T cell types whereas weak agonists only affected differentiated cells. Proliferation of PRS-mutant naïve mouse T cells does not differ from wild-type CD3 ϵ in response to strong agonists. The responsiveness may correlate with the ability of the agonist to elicit a CD3 ϵ conformational change as measured by the ability of CD3 ϵ to bind Nck. The mutation of the CD3 ϵ proline motif and the ITAM significantly impairs the response to weak antigens in differentiated but not naïve cells. Therefore, the importance of Nck-mediate T cell activation is still a matter of debate in terms of various stages of T cell development as well as types of antigenic stimuli.

Clinical implications

Previous studies have suggested the necessary role of Nck in the activation and function of human T cells. The information obtained help to understand, at least in part, the involvement of Nck in the control of T cells responses. The subsequent applications may contribute to finding a new approach for diagnosis or treatment in immunopathologic conditions. Recently, based on the blockage of TCR-Nck interaction, synthetic peptides that mimic the sequence of PRS have been demonstrated to block the Nck binding to CD3 ϵ and the activation of T cells can then be inhibited (Alarcon, et al., 2012). Nck recruitment to the TCR is required to elicit a protective adaptive immune response to tumor antigens in vivo and to allow T cell proliferation in vitro and in vivo (Borroto, et al., 2014). Understanding the role of

adaptor molecule such as Nck would provide a novel approach to the development of specific immunosuppressive or immunomodulatory agents with fewer side effects for the treatment of diseases that are associated with the activation of T cells such as autoimmune diseases and diseases that are associated with rejection of allotransplants or xenotransplants of organs or tissues.

Conclusions

Nck is proposed to play an essential role in T cell activation. Two different models for the association of Nck with TCR/CD3 complex have been proposed. In an ITAM-requiring pathway, Nck is recruited to the membrane proximal site via interaction with SLP-76, forming the activation complex that is involved in actin cytoskeletal rearrangement and T cell activation. In a non-ITAM-requiring pathway, Nck directly binds to a PRS in CD3 ϵ that exposes due to a conformational change in the TCR complex after TCR ligation. Nck recruits associated regulatory proteins to CD3 ϵ and this event displays an alternative way for T cell activation. Therefore, Nck could be a candidate for development of immunosuppressive drugs for treatment of immunopathologic conditions in the future.

Acknowledgements

This work was supported by Naresuan University Research Grant (P2557B024: fiscal year 2014).

References

- Alarcon, B., Ramirez, A., Carmen, A., Luz, M., Borroto, A., & Morreale, A. (2012). Immunosuppressor based on the blockage of TCR-Nck interaction. Patent application number: 20120135041. N.P.: n.p.



- Alberola-Ila, J., Takaki, S., Kerner, J. D., & Perlmutter, R. M. (1997). Differential signaling by lymphocyte antigen receptors. *Annual Review of Immunology*, 15, 125–154.
- Blasutig, I. (2008). Phosphorylated motif recognition and mechanisms of cell signaling in actin-cytoskeleton regulation. Doctoral dissertation, University of Toronto.
- Bokoch, G. M., Wang, Y., Bohl, B. P., Sells, M. A., Quilliam, L. A., & Knaus, U. G. (1996). Interaction of the Nck adapter protein with p21-activated kinase (PAK1). *Journal of Biological Chemistry*, 271, 25746–25749.
- Borroto, A., Arellano, I., Blanco, R., Fuentes, M., Orfao, A., Dopfer, E. P., et al. (2014). Relevance of Nck-CD3 epsilon interaction for T cell activation in vivo. *Journal of Immunology*, 192, 2042–2053.
- Borroto, A., Lama, J., Niedergang, F., Utry-Varsat, A., Alarcon, B., & Alcover, A. (1999). The CD3 epsilon subunit of the TCR contains endocytosis signals. *Journal of Immunology*, 163, 25–31.
- Bubeck-Wardenburg, J., Pappu, R., Bu, J. Y., Mayer, B., Chernoff, J., Straus, D., et al. (1998). Regulation of PAK activation and the T cell cytoskeleton by the linker protein SLP-76. *Immunity*, 9, 607–616.
- Buday, L. (1999). Membrane-targeting of signalling molecules by SH2/SH3 domain-containing adaptor proteins. *Biochimica et Biophysica Acta*, 1422, 187–204.
- Buday, L., Wunderlich, L., & Tamas, P. (2002). The Nck family of adapter proteins: regulators of actin cytoskeleton. *Cellular Signalling*, 14, 723–731.
- Bunnell, S. C., Hong, D. I., Kardon, J. R., Yamazaki, T., McGlade, C. J., Barr, V. A., et al. (2002). T cell receptor ligation induces the formation of dynamically regulated signaling assemblies. *Journal of Cell Biology*, 158, 1263–1275.
- Chen, M., She, H., Kim, A., Woodley, D. T., & Li, W. (2000). Nck β adapter regulates actin polymerization in NIH 3T3 fibroblasts in response to platelet-derived growth factor bb. *Molecular and Cellular Biology*, 20, 7867–7880.
- Chou, M. M., Fajardo, J. E., & Hanafusa, H. (1992). The SH2- and SH3-containing Nck protein transforms mammalian fibroblasts in the absence of elevated phosphotyrosine levels. *Molecular and Cellular Biology*, 12, 5834–5842.
- Dave, V. P., Cao, Z., Browne, C., Alarcon, B., Fernandez-Miguel, G., Lafaille, J., et al. (1997). CD3 δ deficiency arrests development of the $\alpha\beta$ but not the $\gamma\delta$ T cell lineage. *The EMBO Journal*, 16, 1360–1370.
- Fischer, K. D., Kong, Y. Y., Nishina, H., Tedford, K., Marengere, L. E., Kozieradzki, I., et al. (1998). Vav is a regulator of cytoskeletal reorganization mediated by the T-cell receptor. *Current Biology*, 8, 554–562.



- Galisteo, M. L., Chernoff, J., Su, Y. C., Skolnik, E. Y., & Schlessinger, J. (1996). The adaptor protein Nck links receptor tyrosine kinases with the serine-threonine kinase Pak1. *Journal of Biological Chemistry*, 271, 20997-21000.
- Gil, D., Schamel, W. W., Montoya, M., Sanchez-Madrid, F., & Alarcon, B. (2002). Recruitment of Nck by CD3 epsilon reveals a ligand-induced conformational change essential for T cell receptor signaling and synapse formation. *Cell*, 109, 901-912.
- Gil, D., Schrum, A. G., Alarcon, B., & Palmer, E. (2005). T cell receptor engagement by peptide-MHC ligands induces a conformational change in the CD3 complex of thymocytes. *Journal of Experimental Medicine*, 201, 517-522.
- Haks, M. C., Krimpenfort, P., Borst, J., & Kruisbeek, A. M. (1998). The CD3 γ chain is essential for development of both the TCR $\alpha\beta$ and TCR $\gamma\delta$ lineages. *The EMBO Journal*, 27, 1871-1882.
- Hu, Q., Milfay, D., & Williams, L. T. (1995). Binding of NCK to SOS and activation of ras-dependent gene expression. *Molecular and Cellular Biology*, 15, 1169-1174.
- Huebner, K., Kastury, K., Druck, T., Salcini, A. E., Lanfrancione, L., Pelicci, G., et al. (1994). Chromosome Locations of Genes Encoding Human Signal Transduction Adapter Proteins, Nck (NCK), Shc (SHC1), and Grb2 (GRB2). *Genomics*, 22, 281-287.
- Janeway, A. J. (1995). Ligands for the T-cell receptor: hard times for avidity models. *Immunology Today*, 16, 223-225.
- Kang, H., Schneider, H., & Rudd, C. E. (2002). Phosphatidylinositol 3-kinase p85 adaptor function in T-cells, Co-stimulation and regulation of cytokine transcription independent of associated p110. *Journal of Biological Chemistry*, 277, 912-921.
- Lehmann, J. M., Riethmuller, G., & Johnson, J. P. (1990). Nck, a melanoma cDNA encoding a cytoplasmic protein consisting of the src homology units SH2 and SH3. *Nucleic Acids Research*, 18, 1048.
- Lettau, M., Pieper, J., & Janssen, O. (2009). Nck adapter proteins: functional versatility in T cells. *Cell Communication and Signaling*, 7, 1-13.
- Li, W., Hu, P., Skolnik, E. Y., Ullrich, A., & Schlessinger, J. (1992). The SH2 and SH3 domain-containing Nck protein is oncogenic and a common target for phosphorylation by different surface receptors. *Molecular and Cellular Biology*, 12, 5824-5833.
- Lin, J., & Weiss, A. (2001). T cell receptor signalling. *Journal of Cell Science*, 114, 243-244.
- Liu, D. (2014). The adaptor protein Crk in immune response. *Immunology and Cell Biology*, 92, 80-89.
- Love, P. E., Shores, E. W., Johnson, M. D., Tremblay M. L., Lee, E. J., Grinberg, A., et al. (1993). T cell development in mice that lack the ζ chain of the T cell antigen receptor complex. *Science*, 261, 918-921.



- McCarty, J. H. (1998). The Nck SH2/SH3 adaptor protein: a regulator of multiple intracellular signal transduction events. *Bioessays*, 20, 913–921.
- Margolis, B., Silvennoinen, O., Comoglio, F., Roonprapunt, C., Skolnik, E., Ulrich, A., et al. (1992). High-efficiency expression/ cloning of epidermal growth factor-receptor-binding proteins with Src homology 2 domains. *Proceedings of the National Academy of Sciences USA*, 89, 8894–8898.
- Meisenhelder, J., & Hunter, T. (1992). The SH2/SH3 domain-containing protein Nck is recognized by certain anti-phospholipase C- γ 1 monoclonal antibodies, and its phosphorylation on tyrosine is stimulated by platelet-derived growth factor and epidermal growth factor treatment. *Molecular and Cellular Biology*, 12, 5843–5856.
- Ngoenkam, J., Paensuwan, P., Preechanukul, K., Khamsri, B., Yiemwattana, I., Beck-García, E., et al. (2014). Non-overlapping functions of Nck1 and Nck2 adaptor proteins in T cell activation. *Cell Communication and Signaling*, 12, 21.
- Nishimura, R., Li, W., Kashishian, A., Mondino, A., Zhou, M., Cooper, J., & Schlessinger, J. (1993). Two signaling molecules share a phosphotyrosine-containing binding site in the platelet-derived growth factor receptor. *Molecular and Cellular Biology*, 13, 6889–6896.
- Noguchi, T., Matozaki, T., Inagaki, K., Tsuda, M., Fukunaga, K., Kitamura, Y., et al. (1999). Tyrosine phosphorylation of p62 (Dok) induced by cell adhesion and insulin: possible role in cell migration. *EMBO Journal*, 18, 1748–1760.
- Nolz, J. C., Nacusi, L. P., Segovis, C. M., Medeiros, R. B., Mitchell, J. S., Shimizu, Y., et al. (2008). The WAVE2 complex regulates T cell receptor signaling to integrins via Abl- and CrkL-C3G-mediated activation of Rap1. *Journal of Cell Biology*, 182, 1231–1244.
- Okada, S., & Pessin, J. E. (1996). Interactions between Src homology (SH) 2/SH3 adaptor proteins and the guanylnucleotide exchange factor SOS are differentially regulated by insulin and epidermal growth factor. *Journal of Biology Chemistry*, 271, 25533–25538.
- Panchal, S. C., Kaiser, D. A., Torres, E., Pollard, T. D., & Rosen, M. K. (2003). A conserved amphipathic helix in WASP/Scar proteins is essential for activation of Arp2/3 complex. *Natural Structural Biology*, 10, 591–598.
- Park, D. (1997). Cloning, sequencing, and overexpression of SH2/SH3 adaptor protein Nck from mouse thymus. *Molecules and Cells*, 7, 231–236.
- Pramatarova, A., Ochalski, P. G., Chen, K., Gropman, A., Myers, S., Min, K. T., et al. (2003). Nck beta interacts with tyrosine-phosphorylated disabled 1 and redistributes in Reelin-stimulated neurons. *Molecular and Cellular Biology*, 23, 7210–7221.
- Ramos Morales, F., Druker, B. J., & Fischer, S. (1994). Vav binds to several SH2/SH3 containing proteins in activated lymphocytes. *Oncogene*, 9, 1917–1923.



- Risueno, R. Mo, Schamel, W. W., & Alarcon, B. (2008). T cell receptor engagement triggers its CD3epsilon and CD3zeta subunits to adopt a compact, locked conformation. *PLOS ONE*, 3, e1747.
- RiveroLezcano, O. M., Marcilla, A., Sameshima, J. H., & Robbins, K. C. (1995). Wiskott-Aldrich syndrome protein physically associates with Nck through Src homology 3 domains. *Molecular and Cellular Biology*, 15, 5725-5731.
- Rohatgi, R., Nollau, P., Ho, H. Y., Kirschner, M. W., & Mayer, B. J. (2001). Nck and phosphatidylinositol 4,5-bisphosphate synergistically activate actin polymerization through the N-WASP-Arp2/3 pathway. *Journal of Biological Chemistry*, 276, 26448-26452.
- Roy, E., Togbe, D., Holdorf, A. D., Trubetskoy, D., Nabti, S., Küblbeck, G., et al. (2010). Nck adaptors are positive regulators of the size and sensitivity of the T-cell repertoire. *Proceedings of the National Academy of Sciences USA*, 107, 15529-15534.
- Rudolph, M. G., & Wilson, I. A. (2002). The specificity of TCR/pMHC interaction. *Current Opinion in Immunology*, 14, 52-65.
- Skolnik, E. Y., Margolis, B., Mohammadi, M., Lowenstein, E., Fischer, R., & Drepps, A. (1991). Cloning of PI3 kinase-associated p85 utilizing a novel method for expression/cloning of target proteins for receptor tyrosine kinases. *Cell*, 65, 83-90.
- Smith-Garvin, J. E., Koretzky, G. A. & Jordan, M. S. (2009). T cell activation. *Annual Review of Immunology*, 27, 591-619.
- Szymczak, A. L., Workman, C. J., Gil, D., Dilioglou, S., Vignali, K. M., Palmer, E., et al. (2005). The CD3epsilon proline-rich sequence, and its interaction with Nck, is not required for T cell development and function. *Journal of Immunology*, 175, 270-275.
- Takeuchi, K., Yang, H., Ng, E., Park, S. Y., Sun, Z. Y., Reinherz, E. L., et al. (2008). Structural and functional evidence that Nck interaction with CD3epsilon regulates T-cell receptor activity. *Journal of Molecular Biology*, 380, 704-716.
- Taylor, P., Tsai, S., Shameli, A., Serra, P., Wang, J., Robbins, S., et al. (2008). The proline-rich sequence of CD3E as an amplifier of low-avidity TCR signaling. *Journal of Immunology*, 181, 243-255.
- Ullrich, A., & Schlessinger, J. (1990). Signal transduction by receptors with tyrosine kinase activity. *Cell*, 61, 203-212.
- Vorobieva, N., Protopopov, A., Protopopova, M., Kashuba, V., Allikmets, R. L., Modi, W., et al. (1995). Localization of human ARF2 and NCK genes and 13 other Not1-linking clones to chromosome 3 by fluorescence in situ hybridization. *Cytogenetics and Cell Genetics*, 68, 91-94.
- Wang, B., Wang, N., Whitehurst, C. E., She, J., Chen, J. & Terhorst, C. (1999). T lymphocyte development in the absence of CD3E or CD3γδζε. *Journal of Immunology*, 162, 88-94.
- Werlen, G., & Palmer, E. (2002). The T-cell receptor signalosome: a dynamic structure with expanding complexity. *Current Opinion in Immunology*, 14, 299-305.



- Wu J, N., & Koretzky, G. A. (2004). The SLP-76 family of adaptor proteins. *Seminars in Immunology*, 16, 379–393.
- Wunderlich, L., Farago, A., Downward, J., & Buday, L. (1999). Association of Nck with tyrosine-phosphorylated SLP-76 in activated T lymphocytes. *European Journal of Immunology*, 29, 1068–1075.
- Wunderlich, L., Goher, A., Farago, A., Downward, J., & Buday, L. (1999) Requirement of multiple SH3 domains of Nck for ligand binding. *Cellular Signalling*, 11, 253–262.
- Yablonski, D., Kane, L. P., Qian, D., & Weiss, A. (1998). A Nck-Pak1 signaling module is required for T-cell receptor-mediated activation of NFAT, but not of JNK. *The EMBO Journal*, 17, 5647–5657.
- Yiemwattana, I., Ngoenkam, J., Paensuwan, P., Kriangkrai, R., Chuenjitkuntaworn, B., & Pongcharoen, S. (2012). Essential role of the adaptor protein Nck1 in Jurkat T cell activation and function. *Clinical and Experimental Immunology*, 167, 99–107.
- Yoder, J., Pham, C., Iizuka, Y. M., Kanagawa, O., Liu, S. K., McGlade, J., et al. (2001). Requirement for the SLP-76 adaptor GADS in T cell development. *Science*, 291, 1987–1991.