SMALL GTPASES
2020, VOL. 11, NO. 6, 385–391
https://doi.org/10.1080/21541248.2018.1536638

Taylor & Francis
Taylor & Francis Group

Check for updates

Regulation of RhoA activation and cell motility by c-Jun N-terminal kinases and Net1

Arzu Ulu and Jeffrey A. Frost

Department of Integrative Biology and Pharmacology, University of Texas Health Science Center at Houston, Houston, TX, USA

ABSTRACT

Jnks are mitogen activated protein kinases that are best known for regulating transcription and apoptotic signaling. However, they also play important roles in controlling cell motility and invasion by phosphorylating many actin and microtubule regulatory proteins. These mechanisms have important implications for normal cell motility as well as cancer metastasis. Jnks are activated by growth factors and cytokines that stimulate cell motility, and this often requires upstream activation of Rho GTPases. Our recent work indicates that Jnks may also regulate Rho GTPase activation. Specifically, we found that Jnk-dependent phosphorylation of the RhoA guanine nucleotide exchange factor (RhoGEF) Net1A promotes its cytosolic accumulation to drive RhoA activation and actin cytoskeletal reorganization. Net1A is unusual among RhoGEFs in that it is sequestered in the nucleus to prevent aberrant RhoA activation. Importantly, Jnk-stimulated cytosolic localization of Net1A is sufficient to stimulate cell motility and extracellular matrix invasion in non-invasive breast cancer cells. Since Net1A expression is critical for cancer cell motility and invasion *in vitro*, and breast cancer metastasis *in vivo*, these data uncover a previously unappreciated regulatory mechanism that may contribute to metastasis in multiple types of cancer.

ARTICLE HISTORY
Received 13 August 2018
Revised 4 October 2018
Accepted 9 October 2018

KEYWORDS
RhoA; Jnk; Net1; actin cytoskeleton; motility; invasion

Introduction

c-Jun N-terminal kinases (Jnks) are Mitogen Activated Protein Kinase (MAPK) family enzymes that are best known for regulating pro-apoptotic signaling and transcriptional activation. However, these kinases clearly play wider roles in the cell, including the regulation of cell motility and invasion. There are three genes in the Jnk family, Jnk1, Jnk2, and Jnk3, which are also known as stress activated MAP kinases (SAPKγ, α, and β, respectively). There are multiple isoforms for each gene. Jnk2 was the first enzyme discovered, where it was purified from rat livers as a cyclohexamide-stimulated, microtubule-associated protein-2 (MAP2) kinase [1]. Soon thereafter, a protein kinase capable of phosphorylating recombinant c-Jun on its N-terminal activating sites S63 and S73 was purified from U937 cells [2]. Concurrently a UV- and Ras-responsive S63/S73 c-Jun kinase was purified from HeLa cells [3]. However, it was not until the cloning of Jnk1 and Jnk2 that it was appreciated that these different kinase activities corresponded to the same family of enzymes [4,5]. Since then it has been shown that a wide variety of stimuli activate Jnks, ranging from growth factors and oncogenes to stress and apoptotic stimuli [6–8].

Like all MAPKs, Jnk activation is controlled by a multi-tiered kinase cascade consisting of Jnk kinases (MKK4 and MKK7), Jnk kinase kinases (a wide variety of enzymes in the MAP3K family), and in some cases enzymes within the MAP4K family (Figure 1) [8]. The existence of a multi-tiered kinase cascade provides for specificity of response to particular stimuli, as well as signal amplification that makes the activation of Jnks very switch-like [9]. In addition, there are a number of scaffolding proteins that co-localize Jnks with specific MAP2Ks and MAK3Ks to increase signaling specificity and speed of activation [7,10]. These scaffolding proteins also serve to limit Jnk activation to specific subcellular locations.

Regulation of cell motility by Jnks during development

Jnks most often regulate transcriptional activity through phosphorylation of transcription factors such as c-Jun, ATF, and others [11]. However, Jnks are also active in the cytoplasm, and many of these substrates are important for controlling cell motility. A role for Jnk in controlling cell movement was first discovered in

CONTACT Jeffrey A. Frost jeffrey.a.frost@uth.tmc.edu Department of Integrative Biology and Pharmacology, University of Texas Health Science Center at Houston, Houston, TX, USA

© 2018 Informa UK Limited, trading as Taylor & Francis Group

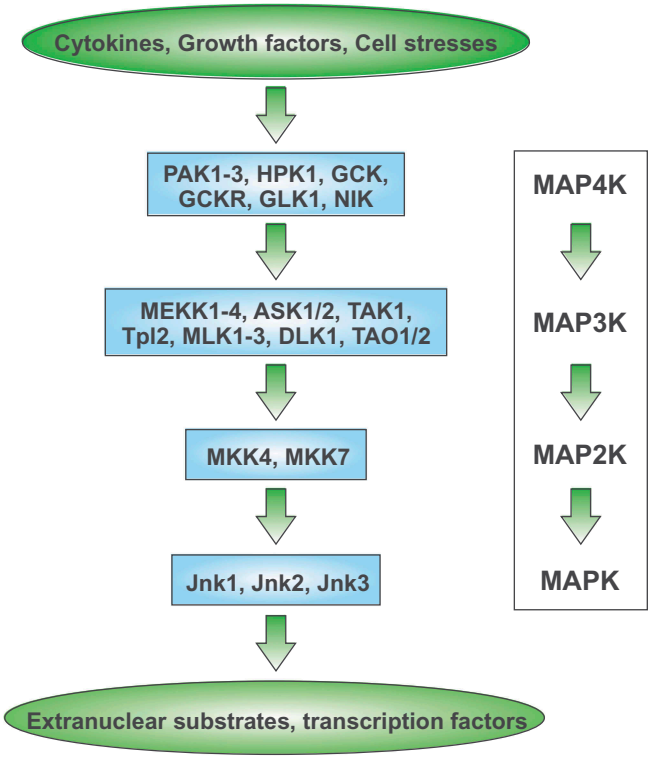

Figure 1. Jnk activating kinase cascades. Jnks are activated by a multi-tiered, sequential kinase cascade that minimally consists of an upstream MAP2K and MAP3K. Some stimuli make use of a MAP4K enzyme. Shown are the genes that have been implicated in Jnk activation within each tier.

Drosophila, where signaling within this cascade was found to be necessary for dorsal closure within the embryo. This developmental process requires epithelial cell sheet spreading, which is mediated by actin cytoskeletal reorganization and cell movement [12]. Specifically, it was found that deletion of either *Hep* (MKK7) or *basket* (Jnk) prevented dorsal closure [13,14]. A later study showed that this was due in part to Jnk-dependent expression of *Dpp* (a BMP homolog), indicating that at least part of the effect was due to transcriptional regulation [15]. Soon thereafter, it was discovered that mice lacking both *Jnk1* and *Jnk2* exhibited a deficit in neural tube closure during fetal development. However, as this process also requires changes proliferation and apoptosis, it was unclear to what extent alterations in cell motility played a role [16,17]. A later study showed that Jnk signaling was important for collective movement of border cells in *Drosophila* egg chambers. In this study, loss of Jnk expression resulted in dissociation of cell clusters with a loss of apical-basal polarity. This was due in part to reduced *D-Paxillin* expression, indicating once again that transcriptional control was an essential aspect of Jnk-regulated cell movement during embryogenesis [18].

Regulation of cell motility by Jnks *in vitro*

Stronger evidence for direct control of cell motility by Jnks comes from studies in cultured cells, where various Jnk substrates regulating cytoskeletal organization have been identified. For example, inhibition of Jnk activity with the small molecule inhibitor SP600125, or by expression of dominant negative Jnk1, blocked motility in fish keratinocytes and rat bladder cancer cells. This was at least partly due to phosphorylation of paxillin on S178, as expression of a mutant paxillin S178A slowed the turnover of focal adhesions and actin stress fibers that is required for optimal cell motility [19]. In a later study, Jnk was shown to phosphorylate the actin bundling protein MARCKSL1 on three sites in neurons and PC3 prostate cancer cells. In this work, Jnk phosphorylation enhanced the actin bundling and filament stabilizing properties of MARCKSL1 [20].

A significant number of studies indicate that Jnk regulates microtubule dynamics by phosphorylating microtubule regulatory proteins. For example, Jnk1 has been shown to phosphorylate the microtubule-associated proteins MAP2 and MAP1B in mouse brains. Mice with *Jnk1* deletion exhibited reduced MAP2 phosphorylation *in vivo* and developed age related neurodegeneration [21]. MAP2 isolated from *Jnk1*$^{-/-}$ mouse brains was also less effective at promoting microtubule polymerization *in vitro*, consistent with its reduced ability to bind to microtubules. In a separate set of studies, expression of dominant negative Jnk1 in mouse embryonic cerebral cortex inhibited neuronal migration *in vivo* and *in vitro*. Moreover, treatment of these neurites with the Jnk inhibitor SP600125 inhibited MAP1B phosphorylation, which was associated with reduced microtubule stability [22,23].

Jnk1 has also been reported to phosphorylate the microtubule binding protein DCX, which is required for interaction with the motor protein Kinesin 1. The ability of Jnk1 to phosphorylate DCX was dependent on its interaction with the Jnk scaffolding protein JIP1, and DCX phosphorylation promoted neurite outgrowth and cell motility in rat primary hippocampal neurons [24]. Jnk1 also interacts with and phosphorylates the microtubule severing protein SGC10/Stathmin2, which prevents its association with microtubules and thus indirectly enhances their stability [25,26]. Jnk1 is also critical for gut elongation in *Xenopus laevis*, where inhibition of Jnk activity with SP610025, or Jnk1 knockdown with morpholinos, inhibited cell-cell adhesion and reduced microtubule stability [27]. Taken together, these results indicate that Jnks regulate microtubule dynamics, cellular adhesion, and actin cytoskeletal organization by

phosphorylating multiple regulatory proteins, and that these events are critical for controlling cell motility and development.

Regulation of Rho protein activity by Jnk

In contrast to the multiple reports demonstrating phosphorylation-dependent regulation of proteins that directly regulate cytoskeletal organization, there are relatively few reports of Jnk directly controlling Rho GTPase activation. One of the only examples is the RhoA GEF p63RhoGEF/GEFT, which is inhibited by interaction with the Jnk-activating MAP3K, MLK3 [28]. In this work it was shown that expression of constitutively active Gαq, or stimulation of the Gq-coupled muscarinic type 1 receptor, promoted interaction between MLK3 and p63RhoGEF, thereby inhibiting its RhoA GDP exchange (GEF) activity. In a feedback loop, this interaction was blocked by phosphorylation of MLK3 by Jnk, thereby releasing p63RhoGEF to allow RhoA activation.

Recently we have reported that Jnk, and to a lesser extent p38 MAPK, can phosphorylate the RhoA GEF Net1A (neuroepithelial cell transforming gene 1A) to promote its cytosolic localization and RhoA activation [29]. Net1A is a RhoA subfamily GEF that regulates breast cancer cell motility *in vitro* [30,31], as well as lung metastasis in a mouse model of breast cancer [32]. Importantly, the ability of Net1 isoforms to stimulate RhoA activation in the cytosol or at the plasma membrane is negatively regulated by nuclear sequestration [33,34]. Net1A localizes to the nucleus because of two nuclear localization signal (NLS) sequences in its N-terminal regulatory domain [35]. Nevertheless, ligand stimulation of quiescent cells causes a rapid export of Net1A from the nucleus that requires Rac1 activation and results in lysine acetylation within the second NLS sequence. This neutralizes the positive charges of the lysines within this NLS, inhibiting its function and most likely slowing the rate of nuclear re-import of Net1A [35,36].

To understand whether additional mechanisms contributed to Net1A cytosolic localization, we tested whether the MAPK family of enzymes played a role. We focused on the Erk, Jnk, and p38 MAPK families as these enzymes are all activated downstream of Rac1 and are known to translocate to the nucleus upon activation [37–41]. We observed that small molecule inhibition of all three MAPK families blocked EGF-stimulated relocalization of Net1A to the cytosol in MCF7 breast cancer cells, but that the cells were most sensitive to inhibition of Jnk family enzymes [29]. Moreover, expression of constitutively active MKK7 or MKK3, which stimulate Jnk and p38 MAPK respectively, was sufficient to maximally stimulate Net1A cytosolic relocalization in the absence of ligand. Jnk-dependent regulation of Net1A subcellular localization occurred following stimulation with TNFα, and also occurred in the invasive breast cancer cell lines BT20, MDA-MB-436, and MBA-MB-453 after stimulation with EGF, Heregulin, or fetal bovine serum. Thus, Jnk-dependent control of Net1A cytosolic accumulation appeared to be a generalizable means of regulation.

To understand how Jnks regulated Net1A cytosolic localization, we tested whether Jnk1 was capable of phosphorylating Net1A. Using purified, recombinant proteins and tandem LC-MS/MS, we showed that Jnk1 phosphorylated Net1A mainly on serine 52 *in vitro*. Importantly, mutation of serine 52 to alanine prevented EGF- or MKK7-stimulated cytosolic relocalization of Net1A. Moreover, a portion of Net1A containing an S52E substitution to mimic Jnk phosphorylation was constitutively localized to the cytosol. To determine whether phosphorylation of this site promoted nuclear export or prevented nuclear import, we treated Net1A transfected cells with the nuclear importin β inhibitor importazole, which promoted cytosolic accumulation of Net1A. The importazole was then washed out and the cells were allowed time to relocalize Net1A to the nucleus. In these experiments, we observed that wild type Net1A completely relocalized to the nucleus within 4 hours of importazole washout, but that Net1A S52E never returned to the nucleus. Taken together these experiments indicated that EGF-stimulated cytosolic relocalization of Net1A required Jnk-dependent phosphorylation of Net1A on serine 52, and that this prevented nuclear re-import of Net1A.

These data still left open the question of how Net1A nuclear export was achieved. Years ago, Schmidt and Hall showed that cytosolic accumulation of an N-terminal truncation mutant of Net1, called Net1ΔN, was blocked by leptomycin B, which is an inhibitor of the nuclear exportin CRM1 [33]. Thus, we tested whether Net1A nuclear export was also CRM1-dependent. We observed that EGF- or MKK7-stimulated Net1A nuclear export was completely blocked by leptomycin B treatment, indicating that CRM1 function was necessary. Interestingly, Net1A lacks a nuclear export signal (NES) sequence, which is normally required for interaction with CRM1. Thus, these data suggest that ligand stimulated nuclear export of Net1A must promote interaction with one or more NES-containing proteins that mediate nuclear exit.

To understand the phenotypic consequences of Net1A regulation by Jnks, we tested for effects on RhoA

activation and actin cytoskeletal reorganization. We found that Net1A S52E substitution did not affect the intrinsic RhoA GEF activity of Net1A, but did allow Net1A to stimulate RhoA activation, myosin light chain phosphorylation, and F-actin accumulation. Thus, cytosolic relocalization was sufficient for Net1A to activate RhoA. We then tested whether this affected cell motility and invasion. For these assays, we used MCF7 cells, which are epithelial in character and thus weakly motile and non-invasive. We found that expression of Net1A S52E significantly stimulated the motility of these cells, and allowed them to invade a Matrigel extracellular matrix (ECM). One mechanism by which cancer cells invade an ECM is through the formation of invadopodia, which are small, metalloproteinase secreting protrusions that allow cells to form pores in a dense ECM [42,43]. MCF7 cells are normally incapable of forming invadopodia. However, we observed that Net1A S52E expression caused these cells to form prominent Tks5-containing invadopodia. Taken together these data indicate that Jnk-stimulated cytosolic relocalization of Net1A in breast cancer cells is sufficient to promote RhoA activation, cell motility, and ECM invasion (Figure 2).

Perspectives

The role of Jnk signaling in cancer cell motility and metastasis is complex. Jnks were originally investigated as kinases that promote tumorigenesis, but they also clearly play a negative role through their pro-apoptotic functions [40,44]. The majority of studies indicate that MKK4 and MKK7 play tumor suppressive roles, as they are mutated in a number of human cancers and suppress metastasis when overexpressed [45,46]. However, MKK4 deletion in human cancer cells has been shown to inhibit metastasis in mice, suggesting that the single allele deletions or mutations that occur in the majority of human cancers underlie a pro-tumorigenic role of low level MKK4/7 signaling [47]. A number of Jnk-directed MAP3Ks have also been shown to contribute to breast cancer invasion and metastasis. MEKK1 deletion impairs metastasis in the MMTV-PyMT mouse model of breast cancer [48]. Similarly, shRNA knockdown of MEKK2 or MLK3 in MDA-MB-231 human breast cancer cells inhibits tumorigenesis and metastasis [49,50]. Alternatively, knockdown of NIK impairs metastasis of colorectal cancer cells in mice [51]. The MAP4K Pak1 may contribute to tumorigenesis and metastasis in a number of human cancers [52]. However, a caveat of these studies is that MAP3K and MAP4K enzymes stimulate multiple signaling pathways in addition to activating Jnks, which undoubtedly contributes to their pro-tumorigenic and pro-metastatic functions.

In regards to Rho GTPase signaling, it is perhaps not surprising that Jnks would also impact Rho GTPase activation. Paks were cloned as Rac- and Cdc42-activated kinases [53], and many MAP3Ks such as MEKKs and MLKs are regulated by Rho proteins [54–56]. Thus, one might expect feedback regulation of Rho GTPase activation. Furthermore, cell motility requires precise

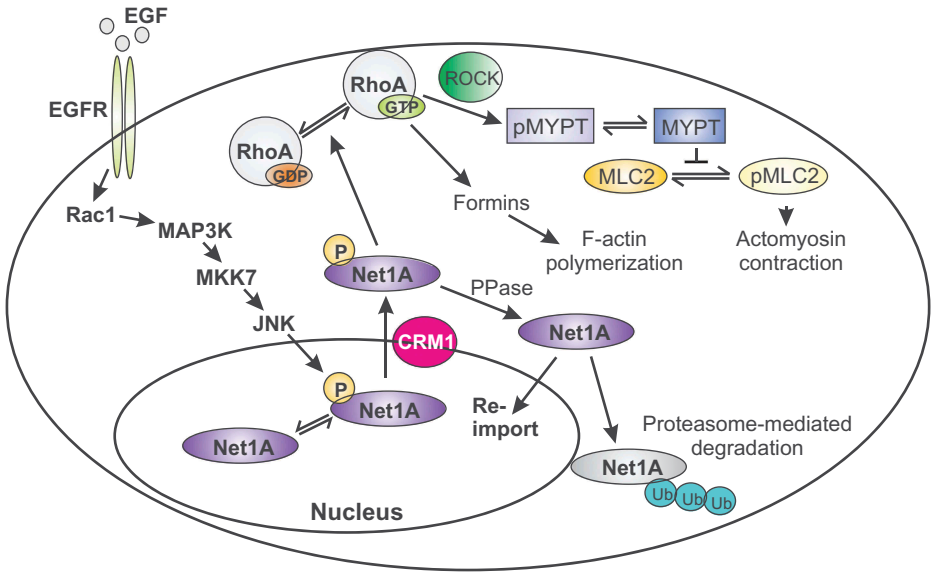

Figure 2. Model for Net1A regulation by Jnk. Extracellular ligands such as EGF promote Net1A cytosolic localization by stimulating CRM1-dependent nuclear export of Net1A and Jnk-dependent phosphorylation of Net1 on serine 52. Jnk phosphorylation of Net1A slows its rate of nuclear re-import to allow for RhoA activation and subsequent actin cytoskeletal reorganization and actomyosin contraction. Cytosolic signaling by Net1A is terminated by dephosphorylation and nuclear re-import, or proteasome-mediated degradation.

regulation of the localization and timing of Rho GTPase activation [57,58], and Jnk-stimulated relocalization of Net1A to the cytosol may represent one such mechanism to promote localized RhoA activity. It might seem counterintuitive that active Rac1 would recruit a RhoA GEF such as Net1A to promote RhoA activation, due to the ability of Rac1 to inhibit RhoA activation [59–62]. However, Rac1 inhibition of RhoA is a dynamic, transient event, so Jnk-dependent relocalization of Net1A may provide a mechanism to maintain a ready pool of Net1A in the correct place to allow for proper timing of RhoA activation. Ultimately, these data may offer an additional mechanism accounting for why small molecule inhibition of Jnk appears to be a potent means to block breast cancer cell invasive capacity [63–65].

How Jnk phosphorylation of Net1A blocks nuclear re-import is an open question. The Jnk phosphorylation site, serine 52, is located between the two NLS sequences in Net1A, and phosphorylation of this site creates a predicted class IV, WW domain binding site, so the simplest explanation is that it drives interaction with a protein that would prevent productive interaction of the NLS sequences with nuclear importins. In regards to mechanisms controlling Net1A subcellular localization, it is still not clear how CRM1 controls Net1A nuclear export. Previously it has been shown that the PH domain of Net1 is sufficient to promote nuclear export [33], so presumably this domain mediates interaction with one or more NES-containing proteins that would respond to ligand stimulation. The identity of these proteins, as well as the signals driving their association, are still open questions. In addition, RhoA is typically activated at the plasma membrane, and mechanisms promoting the association of Net1A with the plasma membrane are not understood. These mechanistic questions are important to answer, as regulation of cell motility and invasion is likely to be an important aspect of how Net1A contributes to breast cancer metastasis [32]. Moreover, a better understanding of these mechanisms may provide novel insights for the design of therapeutic avenues to intervene in the metastatic process.

Acknowledgments

The authors would like to thank members of the Frost Lab for thoughtful discussions. The work discussed here was supported by the National Cancer Institute (grant CA172129 to J.A.F.) and a postdoctoral fellowship from the Cancer Prevention and Research Institute of Texas, UT Health Innovation in Cancer Prevention Research Training Program (grant RP160015 to A.U.).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Cancer Prevention and Research Institute of Texas [RP160015];National Cancer Institute [CA172129];

References

[1] Kyriakis JM, Avruch J. pp54 microtubule-associated protein 2 kinase. A novel serine/threonine protein kinase regulated by phosphorylation and stimulated by poly-L-lysine. J Biol Chem. 1990;265(28):17355–17363. PubMed PMID: 2170374.

[2] Adler V, Polotskaya A, Wagner F, et al. Affinity-purified c-Jun amino-terminal protein kinase requires serine/threonine phosphorylation for activity. J Biol Chem. 1992;267(24):17001–17005. PubMed PMID: 1324919.

[3] Hibi M, Lin A, Smeal T, et al. Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. Genes Dev. 1993;7(11):2135–2148. PubMed PMID: 8224842.

[4] Kyriakis JM, Banerjee P, Nikolakaki E, et al. The stress-activated protein kinase subfamily of c-Jun kinases. Nature. 1994;369(6476):156–160. PubMed PMID: 8177321. .

[5] Derijard B, Hibi M, Wu IH, et al. JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. Cell. 1994;76(6):1025–1037. PubMed PMID: 8137421.

[6] Karin M, Gallagher E. From JNK to pay dirt: jun kinases, their biochemistry, physiology and clinical importance. IUBMB Life. 2005;57(4–5):283–295. PubMed PMID: 16036612. .

[7] Weston CR, Davis RJ. The JNK signal transduction pathway. Curr Opin Cell Biol. 2007;19(2):142–149. PubMed PMID: 17303404. .

[8] Kyriakis JM, Avruch J. Mammalian MAPK signal transduction pathways activated by stress and inflammation: a 10-year update. Physiol Rev. 2012;92(2):689–737. PubMed PMID: 22535895. .

[9] Bagowski CP, Ferrell JE Jr. Bistability in the JNK cascade. Curr Biology. 2001;11(15):1176–1182. PubMed PMID: 11516948.

[10] Engstrom W, Ward A, Moorwood K. The role of scaffold proteins in JNK signalling. Cell Prolif. 2010;43(1):56–66. PubMed PMID: 19922489. .

[11] Bogoyevitch MA, Kobe B. Uses for JNK: the many and varied substrates of the c-Jun N-terminal kinases. Microbiology Molecular Biology Reviews: MMBR. 2006;70(4):1061–1095. PubMed PMID: 17158707; PubMed Central PMCID: PMCPMC1698509. .

[12] Young PE, Richman AM, Ketchum AS, et al. Morphogenesis in Drosophila requires nonmuscle myosin heavy chain function. Genes Dev. 1993;7(1):29–41. PubMed PMID: 8422986.

[13] Glise B, Bourbon H, Noselli S. hemipterous encodes a novel Drosophila MAP kinase kinase, required for epithelial cell sheet movement. Cell. 1995;83(3):451–461. PubMed PMID: 8521475.
[14] Riesgo-Escovar JR, Jenni M, Fritz A, et al. The Drosophila Jun-N-terminal kinase is required for cell morphogenesis but not for DJun-dependent cell fate specification in the eye. Genes Dev. 1996;10(21):2759–2768. PubMed PMID: 8946916.
[15] Riesgo-Escovar JR, Hafen E. Drosophila Jun kinase regulates expression of decapentaplegic via the ETS-domain protein Aop and the AP-1 transcription factor DJun during dorsal closure. Genes Dev. 1997;11(13):1717–1727. PubMed PMID: 9224720.
[16] Sabapathy K, Jochum W, Hochedlinger K, et al. Defective neural tube morphogenesis and altered apoptosis in the absence of both JNK1 and JNK2. Mech Dev. 1999;89(1–2):115–124. PubMed PMID: 10559486.
[17] Kuan CY, Yang DD, Samanta Roy DR, et al. The Jnk1 and Jnk2 protein kinases are required for regional specific apoptosis during early brain development. Neuron. 1999;22(4):667–676. PubMed PMID: 10230788.
[18] Llense F, Martin-Blanco E. JNK signaling controls border cell cluster integrity and collective cell migration. Curr Biology. 2008;18(7):538–544. PubMed PMID: 18394890. .
[19] Huang C, Rajfur Z, Borchers C, et al. JNK phosphorylates paxillin and regulates cell migration. Nature. 2003;424(6945):219–223. PubMed PMID: 12853963. .
[20] Bjorkblom B, Padzik A, Mohammad H, et al. c-Jun N-terminal kinase phosphorylation of MARCKSL1 determines actin stability and migration in neurons and in cancer cells. Mol Cell Biol. 2012;32(17):3513–3526. PubMed PMID: 22751924; PubMed Central PMCID: PMCPMC3421996. .
[21] Chang L, Jones Y, Ellisman MH, et al. JNK1 is required for maintenance of neuronal microtubules and controls phosphorylation of microtubule-associated proteins. Dev Cell. 2003;4(4):521–533. PubMed PMID: 12689591.
[22] Kawauchi T, Chihama K, Nabeshima Y, et al. The in vivo roles of STEF/Tiam1, Rac1 and JNK in cortical neuronal migration. Embo J. 2003;22(16):4190–4201. PubMed PMID: 12912917; PubMed Central PMCID: PMCPMC175802.
[23] Kawauchi T, Chihama K, Nishimura YV, et al. MAP1B phosphorylation is differentially regulated by Cdk5/p35, Cdk5/p25, and JNK. Biochem Biophys Res Commun. 2005;331(1):50–55. PubMed PMID: 15845356.
[24] Gdalyahu A, Ghosh I, Levy T, et al. DCX, a new mediator of the JNK pathway. Embo J. 2004;23(4):823–832. PubMed PMID: 14765123; PubMed Central PMCID: PMCPMC380994.
[25] Tararuk T, Ostman N, Li W, et al. JNK1 phosphorylation of SCG10 determines microtubule dynamics and axodendritic length. J Cell Biol. 2006;173(2):265–277. PubMed PMID: 16618812; PubMed Central PMCID: PMCPMC2063817.
[26] Westerlund N, Zdrojewska J, Padzik A, et al. Phosphorylation of SCG10/stathmin-2 determines multipolar stage exit and neuronal migration rate. Nat Neurosci. 2011;14(3):305–313. PubMed PMID: 21297631.
[27] Dush MK, Nascone-Yoder NM. Jun N-terminal kinase maintains tissue integrity during cell rearrangement in the gut. Development. 2013;140(7):1457–1466. PubMed PMID: 23462475; PubMed Central PMCID: PMCPMC3596989.
[28] Swenson-Fields KI, Sandquist JC, Rossol-Allison J, et al. MLK3 limits activated Galphaq signaling to Rho by binding to p63RhoGEF. Mol Cell. 2008;32(1):43–56. PubMed PMID: 18851832; PubMed Central PMCID: PMCPMC2603627.
[29] Ulu A, Oh W, Zuo Y, et al. Stress-activated MAPKs and CRM1 regulate the subcellular localization of Net1A to control cell motility and invasion. J Cell Sci. 2018;131(3). PubMed PMID: 29361525; PubMed Central PMCID: PMCPMC5826041. DOI:10.1242/jcs.204644.
[30] Murray D, Horgan G, Macmathuna P, et al. NET1-mediated RhoA activation facilitates lysophosphatidic acid-induced cell migration and invasion in gastric cancer. Br J Cancer. 2008;99(8):1322–1329. PubMed PMID: 18827818; PubMed Central PMCID: PMCPMC2570507.
[31] Carr HS, Zuo Y, Oh W, et al. Regulation of focal adhesion kinase activation, breast cancer cell motility, and amoeboid invasion by the RhoA guanine nucleotide exchange factor Net1. Mol Cell Biol. 2013;33(14):2773–2786. PubMed PMID: 23689132; PubMed Central PMCID: PMCPMC3700125.
[32] Zuo Y, Ulu A, Chang JT, et al. Contributions of the RhoA guanine nucleotide exchange factor Net1 to polyoma middle T antigen-mediated mammary gland tumorigenesis and metastasis. Breast Cancer Res. 2018;20(1):41. 10.1186/s13058-018-0966-2. PubMed PMID: 29769144; PubMed Central PMCID: PMCPMC5956559.
[33] Schmidt A, Hall A. The Rho exchange factor Net1 is regulated by nuclear sequestration. J Biol Chem. 2002;277(17):14581–14588. PubMed PMID: 11839749.
[34] Qin H, Carr HS, Wu X, et al. Characterization of the biochemical and transforming properties of the neuroepithelial transforming protein 1. J Biol Chem. 2005;280(9):7603–7613. PubMed PMID: 15611121.
[35] Song EH, Oh W, Ulu A, et al. Acetylation of the RhoA GEF Net1A controls its subcellular localization and activity. J Cell Sci. 2015;128(5):913–922. PubMed PMID: 25588829; PubMed Central PMCID: PMCPMC4342578.
[36] Carr HS, Morris CA, Menon S, et al. Rac1 controls the subcellular localization of the Rho guanine nucleotide exchange factor Net1A to regulate focal adhesion formation and cell spreading. Mol Cell Biol. 2013;33(3):622–634. PubMed PMID: 23184663; PubMed Central PMCID: PMCPMC3554212.
[37] Minden A, Lin A, Claret FX, et al. Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. Cell. 1995;81(7):1147–1157. PubMed PMID: 7600582.
[38] Frost JA, Steen H, Shapiro P, et al. Cross-cascade activation of ERKs and ternary complex factors by Rho family proteins. Embo J. 1997;16(21):6426–6438. PubMed PMID: 9351825; PubMed Central PMCID: PMCPMC1170249.

[39] Sever R, Brugge JS. Signal transduction in cancer. Cold Spring Harb Perspect Med. 2015;5(4). PubMed PMID: 25833940; PubMed Central PMCID: PMCPMC4382731. DOI:10.1101/cshperspect.a006098.
[40] Ebelt ND, Cantrell MA, Van Den Berg CL. c-Jun N-Terminal Kinases Mediate a Wide Range of Targets in the Metastatic Cascade. Genes Cancer. 2013;4(9–10):378–387. 10.1177/1947601913485413. PubMed PMID: 24349635; PubMed Central PMCID: PMCPMC3863335.
[41] Wagner EF, Nebreda AR. Signal integration by JNK and p38 MAPK pathways in cancer development. Nature Reviews Cancer. 2009;9(8):537–549. PubMed PMID: 19629069.
[42] Eckert MA, Yang J. Targeting invadopodia to block breast cancer metastasis. Oncotarget. 2011;2(7):562–568. PubMed PMID: 21725138; PubMed Central PMCID: PMCPMC3248174.
[43] Parekh A, Weaver AM. Regulation of invadopodia by mechanical signaling. Exp Cell Res. 2016;343(1):89–95. PubMed PMID: 26546985; PubMed Central PMCID: PMCPMC4851576.
[44] Whitmarsh AJ, Davis RJ. Role of mitogen-activated protein kinase kinase 4 in cancer. Oncogene. 2007;26 (22):3172–3184. PubMed PMID: 17496914.
[45] Teng DH, Perry WL 3rd, Hogan JK, et al. Human mitogen-activated protein kinase kinase 4 as a candidate tumor suppressor. Cancer Res. 1997;57(19):4177–4182. PubMed PMID: 9331070.
[46] Vander Griend DJ, Kocherginsky M, Hickson JA, et al. Suppression of metastatic colonization by the context-dependent activation of the c-Jun NH2-terminal kinase kinases JNKK1/MKK4 and MKK7. Cancer Res. 2005;65(23):10984–10991. PubMed PMID: 16322247.
[47] Cunningham SC, Gallmeier E, Hucl T, et al. Targeted deletion of MKK4 in cancer cells: a detrimental phenotype manifests as decreased experimental metastasis and suggests a counterweight to the evolution of tumor-suppressor loss. Cancer Res. 2006;66(11):5560–5564. PubMed PMID: 16740690.
[48] Cuevas BD, Winter-Vann AM, Johnson NL, et al. MEKK1 controls matrix degradation and tumor cell dissemination during metastasis of polyoma middle-T driven mammary cancer. Oncogene. 2006;25(36):4998–5010. PubMed PMID: 16568086.
[49] Cronan MR, Nakamura K, Johnson NL, et al. Defining MAP3 kinases required for MDA-MB-231 cell tumor growth and metastasis. Oncogene. 2012;31(34):3889–3900. PubMed PMID: 22139075; PubMed Central PMCID: PMCPMC3297722.
[50] Chen J, Gallo KA. MLK3 regulates paxillin phosphorylation in chemokine-mediated breast cancer cell migration and invasion to drive metastasis. Cancer Res. 2012;72(16):4130–4140. PubMed PMID: 22700880.
[51] Qin M, Zhang J, Xu C, et al. Knockdown of NIK and IKKbeta-Binding Protein (NIBP) Reduces Colorectal Cancer Metastasis through Down-Regulation of the Canonical NF-kappaBeta Signaling Pathway and Suppression of MAPK Signaling Mediated through ERK and JNK. PloS one. 2017;12(1):e0170595. PubMed PMID: 28125661; PubMed Central PMCID: PMCPMC5268490 article.
[52] Kumar R, Li DQ. PAKs in human cancer progression: from inception to cancer therapeutic to future oncobiology. Adv Cancer Res. 2016;130:137–209. PubMed PMID: 27037753.
[53] Manser E, Leung T, Salihuddin H, et al. A brain serine/threonine protein kinase activated by Cdc42 and Rac1. Nature. 1994;367(6458):40–46. PubMed PMID: 8107774.
[54] Fanger GR, Johnson NL, Johnson GL. MEK kinases are regulated by EGF and selectively interact with Rac/Cdc42. Embo J. 1997;16(16):4961–4972. PubMed PMID: 9305638; PubMed Central PMCID: PMCPMC1170131.
[55] Gallagher ED, Gutowski S, Sternweis PC, et al. RhoA binds to the amino terminus of MEKK1 and regulates its kinase activity. J Biol Chem. 2004;279(3):1872–1877. PubMed PMID: 14581471.
[56] Gallo KA, Johnson GL. Mixed-lineage kinase control of JNK and p38 MAPK pathways. Nature Reviews Molecular Cell Biology. 2002;3(9):663–672. PubMed PMID: 12209126.
[57] Machacek M, Hodgson L, Welch C, et al. Coordination of Rho GTPase activities during cell protrusion. Nature. 2009;461(7260):99–103. PubMed PMID: 19693013; PubMed Central PMCID: PMCPMC2885353.
[58] Martin K, Reimann A, Fritz RD, et al. Spatio-temporal co-ordination of RhoA, Rac1 and Cdc42 activation during prototypical edge protrusion and retraction dynamics. Sci Rep. 2016;6:21901. PubMed PMID: 26912264; PubMed Central PMCID: PMCPMC4766498.
[59] Sander EE, Ten Klooster JP, van Delft S, et al. Rac downregulates Rho activity: reciprocal balance between both GTPases determines cellular morphology and migratory behavior. J Cell Biol. 1999;147(5):1009–1022. PubMed PMID: 10579721; PubMed Central PMCID: PMCPMC2169355.
[60] Nimnual AS, Taylor LJ, Bar-Sagi D. Redox-dependent downregulation of Rho by Rac. Nat Cell Biol. 2003;5 (3):236–241. PubMed PMID: 12598902.
[61] Zenke FT, Krendel M, DerMardirossian C, et al. p21-activated kinase 1 phosphorylates and regulates 14-3-3 binding to GEF-H1, a microtubule-localized Rho exchange factor. J Biol Chem. 2004;279(18):18392–18400. PubMed PMID: 14970201.
[62] Alberts AS, Qin H, Carr HS, et al. PAK1 negatively regulates the activity of the Rho exchange factor NET1. J Biol Chem. 2005;280(13):12152–12161. PubMed PMID: 15684429.
[63] Tang ZN, Zhang F, Tang P, et al. RANKL-induced migration of MDA-MB-231 human breast cancer cells via Src and MAPK activation. Oncol Rep. 2011;26 (5):1243–1250. PubMed PMID: 21725611.
[64] Mitra S, Lee JS, Cantrell M, et al. c-Jun N-terminal kinase 2 (JNK2) enhances cell migration through epidermal growth factor substrate 8 (EPS8). J Biol Chem. 2011;286(17):15287–15297. PubMed PMID: 21357683; PubMed Central PMCID: PMCPMC3083186.
[65] Zhuang X, Zhang H, Li X, et al. Differential effects on lung and bone metastasis of breast cancer by Wnt signalling inhibitor DKK1. Nat Cell Biol. 2017;19 (10):1274–1285. PubMed PMID: 28892080.