Department of Basic Medical Sciences

Division of Molecular Cell Signaling
分子細胞情報分野

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Protein phosphorylation and dephosphorylation are among the most important intracellular signaling mechanisms, and are mediated, respectively, by protein kinases and protein phosphatases. We study various aspects of cellular signal transduction with a particular emphasis on the role and regulation of protein phosphorylation and dephosphorylation in cellular stress responses, using yeast cells.

1. Hog1 MAPK is a cytoplasmic osmosensor that governs the fidelity and dynamics of the HOG pathway

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Signaling by the conserved mitogen-activated protein kinase (MAPK) family is a major cellular mechanism through which eukaryotic cells respond to various extracellular stimuli. All MAPKs are activated through a three-tier kinase cascade, composed of a MAPK, a MAPK kinase (MAPKK) and a MAPKK kinase (MAPKKK). Distinct MAPKKKs activated by a specific stimulus phosphorylate and thus activate a cognate MAPKK, which then phosphorylates and activates a downstream MAPK. Activated MAPKs regulate pertinent adaptive responses, such as gene expression, cell cycle progression, and apoptosis. There are several subfamilies of MAPKs both in higher and lower eukaryotes.

Extreme osmotic environments are major threats to living organisms. To cope with external high osmolarity, the budding yeast *Saccharomyces cerevisiae* activates the Hog1 MAPK through the high osmolarity glycerol (HOG) signaling pathway. Yeast achieves long-term adaptation to hyper-osmotic conditions by accumulating the compatible osmolyte glycerol in the cytoplasm. To do so, activated Hog1 is transported from the cytoplasm to the nucleus, where it induces the expression of the genes that encode enzymes necessary for glycerol synthesis (Gpd1, Gpp1/2, etc), and the gene that encodes glycerol/proton symporter Stl1. In the cytoplasm, activated Hog1 closes the glycerol leak channel Fps1. Thus, Hog1 enhances the production, import, and retention of glycerol. Activated Hog1 also regulates cell cycle progression for optimum adaptation.

The HOG pathway comprises the upstream SLN1 and SHO1 branches, both of which activate the Hog1 MAPK. For activation of the HOG pathway, an osmosensor must detect extracellular osmotic change and subsequently transduce a signal to the cytoplasm. The Sln1 sensor histidine kinase has been firmly established as the osmosensor for the SLN1 branch. In the SNL1 branch, the Sln1-Ypd1-Ssk1 phospho-relay activates the functionally redundant MAPKKK Ssk2 and Ssk22 (Ssk2/22). The SHO1 branch is further divided into SHO1(H) and SHO1(M) sub-branches that respectively employ the related osmosensing complexes Sho1/Opy2/Hkr1 and Sho1/Opy2/Msb2. Both SHO1(H) and SHO1(M) activate the MAPK kinase kinase (MAPKKK)

Ste11. Both Ste11 and Ssk2/22 activate the MAPK kinase (MAPKK) Pbs2, which then activates Hog1.

This year, we found that osmotic activation of Hog1 requires, in addition to the previously identified transmembrane osmosensors (Sho1/Opy2/Hkr1, Sho1/Opy2/Msb2 and Sln1), a fourth, cytoplasmic osmosensor OS4, which is mediated by Hog1 itself. The properties of a constitutively-active OS4 mutant suggest a two-step activation mechanism, in which Hog1 is first primed by osmostress for phosphorylation by Pbs2. OS4 also prevents accidental noisy activation of Hog1, and inhibits mating-to-Hog1 crosstalk. OS4 improves the efficiency of Hog1 activation at very high osmolarity (>1.2 M NaCl). Through experiments and model construction, we showed that complex activation dynamics of Hog1 are generated by simple interplay between osmosensors and glycerol accumulation.

2. Increased interaction between osmosensor Sho1 and membrane anchor Opy2 activates the SHO1 branch of the osmoregulatory HOG MAPK pathway

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The SHO1 branch, which is one of the upstream branches of osmoregulatory HOG signaling pathway, employs two related but distinct signaling mechanisms, which are called the SHO1(H) and SHO1(M) sub-branches that respectively utilize the related osmosensing complexes Sho1/Opy2/Hkr1 and Sho1/Opy2/Msb2. Sho1 is composed of four transmembrane (TM) domains and a cytoplasmic SH3 domain that binds to the MAPK kinase (MAPKK) Pbs2. Last year, we demonstrated that Sho1 is an osmosensor in the SHO1 branch of the HOG pathway. Sho1 forms planar oligomers of the dimers-of-trimers architecture by dimerizing at the TM1/TM4 interface and trimerizing at the TM2/TM3 interface. High external osmolarity induces not only structural changes in the Sho1 TM domains, but also the binding between Sho1 and the cytoplasmic adaptor protein Ste50. This Sho1-Ste50 interaction leads to Hog1 activation. In addition to its osmosensing function, Sho1 oligomer serves as a scaffold by binding to the transmembrane protein Opy2 at the TM1/TM4 interface, and to another membrane protein Hkr1/Msb2 at the TM2/TM3 interface. Opy2 is a single-path TM protein of 360 amino acids. Its cytoplasmic region contains three (two major and one minor) Ste50 binding sites that are collectively essential for signaling in the SHO1 branch. As Ste50 is constitutively bound to Ste11, Opy2 recruits the Ste50/Ste11 complex to the plasma membrane. Membrane localization of Ste50 is an important function of Opy2, as an artificial membrane localization of Ste50, by for example using the C-terminal prenylation site of Ras2 (Cpr), suppresses the Hog1 activation defect of *opy2*Δ mutation.

In response to hyperosmolarity, the SHO1 branch activates Hog1 through the Ste20-Ste11-Pbs2-Hog1 kinase cascade. The PAK-like kinase Ste20 is recruited to the membrane by the small G protein Cdc42 as well as by Hkr1 (probably through a hypothesized adaptor protein) or Msb2 through a Bem1 adaptor protein. Similarly, the MAPKK kinase (MAPKKK) Ste11 is recruited to the membrane by the Opy2-Ste50 complex. Ste50 is a cytoplasmic adaptor protein that binds both to Ste11 and to the single-path membrane anchor protein Opy2. Finally, Pbs2 is also recruited to the membrane by Sho1. Thus, both the Ste20→Ste11 reaction and the Ste11→Pbs2 reaction take place on the membrane. One or both of these activation reactions are likely regulated by osmostress; however, no such mechanisms were known.

This year, we studied the functional roles of the interaction between Sho1 and Opy2 in the activation of the SHO1 branch, especially at the process of the Ste11→Pbs2 reaction. We isolated several constitutively-active Sho1 mutants, which harbor the mutation within the TM region. Co-precipitation experiments indicated that one of the active mutations named A/D increased the affinity between the TM domains of Sho1 and Opy2, while introduction of the mutations, which suppressed the increased affinity, into Opy2 abrogated the constitutive activation of the SHO1 branch by Sho1-A/D. In addition, the A/D mutation enhanced the activation of the SHO1 branch as well as the Sho1-Opy2 affinity synergistically with the Opy2-A104V mutation, which strengthens the Sho1-Opy2 interaction. These results indicate that increased interaction of the TM regions of Sho1 and Opy2 leads to the activation of the SHO1 branch, suggesting the possibility that high osmotic stress enhances the Sho1-Opy2 affinity to promote the phosphorylation of Pbs2 by Ste11 by bringing them together.

3. A scaffold protein Ahk1 that associates with Hkr1, Sho1, Ste11 and Pbs2 inhibits crosstalk signaling from the Hkr1 osmosensor to the Kss1 MAPK

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The SHO1 branch involves two putative osmosensors Hkr1 and Msb2, in addition to the aforementioned Sho1 osmosensor. Hkr1 and Msb2 share a common function, as it is necessary to disrupt both the *HKR1* and *MSB2* genes to completely inac-

tivate the SHO1 branch. Both Hkr1 and Msb2 are single-path TM proteins whose extracellular domains contain a highly O-glycosylated Ser/Thr-rich (STR) domain and a conserved Hkr1-Msb2 homology (HMH) domain. Deletion of the STR domain from either Hkr1 or Msb2 constitutively activates the protein, whereas deletion of the HMH domain inactivates the protein, suggesting that both Hkr1 and Msb2 are involved in signaling. In spite of their similar extracellular domains, Hkr1 and Msb2 have completely different cytoplasmic regions. The cytoplasmic region of Msb2 functionally interacts with the scaffold protein Bem1, which binds, among other proteins, Ste20 and the Ste20-activating protein Cdc42. Thus, Msb2 and Bem1 help activate Ste20 on the plasma membrane. The interaction between Msb2 and Opy2 then brings Ste11 to the activated Ste20 on the membrane, thereby activating Ste11 by phosphorylation. In contrast to Msb2, little is yet known about the signaling role of the cytoplasmic domain of Hkr1 (Hkr1-cyto).

This year, we showed that Hkr1-cyto likely helps Hog1 activation by a mechanism different from that employed by Msb2-cyto. We found that deletion of Hkr1-cyto only partially inhibited osmostress-induced Hog1 activation. However, the same deletion mutation completely inhibited Hog1 activation by constitutively-active mutants of the Hog1 pathway signaling molecules Opy2 or Ste50, indicating that Hkr1-cyto participates in certain aspect of Hog1 activation.

Next, using a mass spectrometric method, we identified a protein, termed Ahk1 (Associated with Hkr1), which binds to Hkr1-cyto. Deletion of the *AHK1* gene (in the absence of other Hog1 upstream branches) only partially inhibited osmostress-induced Hog1 activation. In contrast, Hog1 could not be activated by constitutively-active mutants of Opy2 or Ste50 in *ahk1*Δ cells, whereas robust Hog1 activation occurred in *AHK1*$^{+}$ cells. Thus, the lack of Ahk1 and the lack of Hkr1-cyto had similar effects on Hog1 activation by osmostress and by constitutively-active proteins. In addition to Hkr1-cyto binding, Ahk1 also bound to other signaling molecules in the SHO1(H) sub-branch, including Sho1, Ste11, and Pbs2. Although osmotic stimulation of Hkr1 does not activate the Kss1 MAPK, deletion of *AHK1* allowed Hkr1 to activate Kss1 by cross-talk. Thus Ahk1 is a scaffold protein in the SHO1(H) sub-branch, and prevents incorrect signal flow from Hkr1 to Kss1.

Publications

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Department of Basic Medical Sciences

Division of Neuronal Network
神経ネットワーク分野

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Our major research interest is the molecular mechanisms of higher brain functions in mammals such as emotion, and learning and memory. We are especially focusing on the roles of functional molecules localized in synapses, for instance, neurotransmitter receptors, signal transduction molecules and adhesion molecules, in neuronal information processing. We are examining receptor functions, synaptic transmission and plasticity, and their roles in the whole animal with electrophysiological, biochemical, molecular genetic and behavioral approaches. We are also trying to elucidate fundamental aspects of psychiatrical and neurological disorders using model animals.

1. The active zone protein CAST regulates synaptic vesicle recycling and quantal size in the mouse hippocampus

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Synaptic efficacy is determined by various factors, including the quantal size, which is dependent on the amount of neurotransmitters in synaptic vesicles at the presynaptic terminal. It is essential for stable synaptic transmission that the quantal size is kept within a constant range and that synaptic efficacy during and after repetitive synaptic activation is maintained by replenishing release sites with synaptic vesicles. However, the mechanisms for these fundamental properties have still been undetermined. We found that the active zone protein CAST (cytomatrix at the active zone structural protein) played pivotal roles in both presynaptic regulation of quantal size and recycling of endocytosed synaptic vesicles. In the CA1 region of hippocampal slices of the CAST knockout mice, miniature excitatory synaptic responses were increased in size and synaptic depression after prolonged synaptic activation was larger, which was attributable to selective impairment of synaptic vesicle trafficking via the endosome in the presynaptic terminal likely mediated by Rab6. Therefore, CAST serves as a key molecule that regulates dynamics and neurotransmitter contents of synaptic vesicles in the excitatory presynaptic terminal in the central nervous system.

2. Emerging roles of ARHGAP33 in intracellular trafficking of TrkB and pathophysiology of neuropsychiatric disorders

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Intracellular trafficking of receptor proteins is essential for neurons to detect various extracellular factors during the formation and refinement of neural circuits. However, the precise mechanisms underlying the trafficking of neurotrophin receptors to synapses remain elusive. In this study, we demonstrate that a brain-enriched sorting nexin, ARHGAP33, is a new type of regulator for the intracellular trafficking of TrkB, a high-affinity receptor for brain-derived neurotrophic factor. ARHGAP33 knockout (KO) mice exhibit reduced expression of synaptic TrkB, impaired spine development and neuropsychiatric disorder-related behavioral abnormalities. These deficits are rescued by specific pharmacological enhancement of TrkB signaling in ARHGAP33 KO mice. Mechanistically, ARHGAP33 interacts with SORT1 to cooperatively regulate TrkB trafficking. Human ARHGAP33 is associated with brain phenotypes and reduced SORT1 expression is found in patients with schizophrenia. We propose that ARHGAP33/SORT1-mediated TrkB trafficking is essential for synapse development and that the dysfunction of this mechanism may be a new molecular pathology of neuropsychiatric disorders.

3. PX-RICS-deficient mice mimic autism spectrum disorder in Jacobsen syndrome through impaired $GABA_A$ receptor trafficking

Fumiko Arima-Yoshida, Tsutomu Nakamura[17], Fumika Sakaue[17], Yukiko Nasu-Nishimura[17], Yasuko Takeda[17], Ken Matsuura[17], Natacha Akshoomoff[18], Sarah N. Mattson[19], Paul D. Grossfeld[20], Tetsu Akiyama[17] and Toshiya Manabe: [17]Laboratory of Molecular and Genetic Information, Institute of Molecular and Cellular Biosciences, University of Tokyo, [18]Department of Psychiatry, School of Medicine, University of California, San Diego, USA, [19]Department of Psychology, San Diego State University, USA, [20]Department of Pediatrics, School of Medicine, University of California, San Diego, USA.

Jacobsen syndrome (JBS) is a rare congenital disorder caused by a terminal deletion of the long arm of chromosome 11. A subset of patients exhibit social behavioral problems that meet the diagnostic criteria for autism spectrum disorder (ASD); however, the underlying molecular pathogenesis remains poorly understood. PX-RICS is located in the chromosomal region commonly deleted in JBS patients with autistic-like behavior. In this study, we report that PX-RICS-deficient mice exhibit ASD-like social behaviors and ASD-related comorbidities. PX-RICS-deficient neurons show reduced surface γ-aminobutyric acid type A receptor ($GABA_AR$) levels and impaired $GABA_AR$-mediated synaptic transmission. PX-RICS, GABARAP and 14-3-3ζ/θ form an adaptor complex that interconnects $GABA_AR$ and dynein/dynactin, thereby facilitating $GABA_AR$ surface expression. ASD-like behavioral abnormalities in PX-RICS-deficient mice are ameliorated by enhancing inhibitory synaptic transmission with a $GABA_AR$ agonist. Our findings demonstrate a critical role of PX-RICS in cognition and suggest a causal link between PX-RICS deletion and ASD-like behavior in JBS patients.

Publications

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Department of Basic Medical Sciences

Division of Cell Signaling and Molecular Medicine
分子シグナル制御分野

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The aims of the ongoing research projects in our laboratory are to elucidate the regulatory mechanisms of intracellular signal transduction systems responsible for cell-fate decisions, such as MAP kinase cascades and Stress granules. Perturbation of these signaling systems is involved in a variety of life-threatening diseases, including cancer, autoimmune diseases, neurodegenerative disorders and type 2 diabetes. Our laboratory also aims to develop new diagnostic or therapeutic tools for currently intractable disorders in which these pathways are involved.

1. TIA1 oxidation inhibits stress granule assembly and sensitizes cells to stress-induced apoptosis.

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When confronted with environmental stresses, cells either activate defense mechanisms to survive or initiate apoptosis, depending on the level and type of stress. One of the major cellular defense mechanisms is the assembly of stress granules (SGs). SGs are highly conserved cytoplasmic ribonucleoprotein foci that appear when eukaryotic cells are exposed to specific types of stress such as endoplasmic reticulum (ER) stress, heat shock, hypoxia, arsenite or viral infection. The core components of SGs are large aggregates of stalled translation pre-initiation complexes that contain mRNA, 40S ribosomal subunits, translation initiation factors and several RNA-binding proteins (RBPs). In general, the assembly of SGs is triggered by stress-induced phosphorylation of eIF2α, and requires self-aggregation of certain RBPs such as TIA1 or G3BP, both of which possess oligomerization domains. In cells under various stresses, eIF2α is phosphorylated by a number of different stress-sensing kinases including PKR. Phosphorylation of eIF2α suppresses productive translation initiation by preventing formation of the eIF2-GTP-Met-tRNAi complex. Under the stress conditions, specific RBPs such as TIA1 or G3BP, instead of the ternary complex, interact with an mRNA in the 43S complex, leading to the assembly of a translationally stalled 48S complex. Self-oligomerization of TIA1 or G3BP then promotes the aggregation of these 48S complexes at discrete cytoplasmic foci termed SGs. Therefore, both RNA binding and self-oligomerization of these RBPs are crucial for the assembly of SGs. Indeed, TIA1 possesses three RNA recognition motifs (RRMs) at the NH_2 terminus, along with a glutamine-rich prion-related domain at the COOH terminus that is responsible for its prion-like self-aggregation.

SGs serve as sites for mRNA storage and triage. While housekeeping mRNAs are recruited to and sequestered into SGs during stress, certain mRNAs encoding proteins involved in stress tolerance (e.g., heat shock proteins or molecular chaperones) are excluded from SGs. Therefore, by assembling SGs, cells temporarily reduce the synthesis of housekeeping proteins to prevent accumulation of mis-

folded proteins, and optimize translation of stress-responsive anti-apoptotic mRNAs. Besides mRNA sorting and translational suppression, SGs sequester several apoptosis regulatory factors into granules and thereby inhibit stress-induced cell death signaling. We have previously reported that when cells are exposed to a SG-inducing stress, the signaling adaptor protein RACK1 is sequestered into SGs, and this sequestration inhibits the SAPK pathways and subsequent apoptosis. Thus, formation of SGs serves as a cellular adaptive defense mechanism and protects cells from apoptosis under adverse conditions, by regulating mRNA translation as well as by sequestering signaling molecules. Although many types of stress have been reported to induce SG formation, the assembly of SGs under multiple stress conditions has not yet been elucidated. Furthermore, little is known about the role of SGs in the development of human diseases.

This year, we revealed that reactive oxygen species (ROS) oxidized the major SG-nucleating protein TIA1 at Cys36 and consequently suppresses SG assembly by impeding the interaction between TIA1 and its target mRNAs. Thus, when cells are confronted with a SG-inducing stress such as ER stress caused by protein misfolding, together with ROS-induced oxidative stress, they cannot form SGs, resulting in the promotion of apoptosis. We also showed that the suppression of SG formation by oxidative stress might underlie the neuronal cell death seen in neurodegenerative diseases. Our data demonstrate for the first time that SG assembly can be inhibited by oxidative stress, and delineate a novel aetiological aspect of oxidative stress. As oxidative stress is induced by diverse pathological insults, ROS-mediated disturbance of SG assembly may be involved in various pathological processes including neurodegeneration.

2. Identification of novel substrates of human mitogen-activated protein kinases.

Ryosuke Naka, Seina Oe, Hisashi Mori-izumi, Sae Uchida, Tsubasa Tokunaga, Takanaori Nakamura, Yuji Kubota, and Mutsuhiro Takekawa

Sequential activation of protein kinases within MAPK cascades is an evolutionary-conserved mechanism of intracellular signaling among eukaryotes. In human cells, at least three functionally distinct subfamilies of MAPKs are present, namely, ERK1/2, JNK1/2/3, and p38α/β/γ/δ. While the classical ERK MAPK is predominantly activated by mitogenic stimuli, two relatively newly identified MAPKs, p38 and JNK, are preferentially activated by various environmental stresses (e.g., ultraviolet-light and γ-irradiation, oxidative stress, DNA-damaging reagents, osmotic stress, and pro-inflammatory cytokines). Therefore, p38 and JNK MAPKs are collectively referred to as stress-activated protein kinases (SAPKs). Each of these MAPK cascades can regulate several different and sometimes overlapping biological functions. In general, the ERK pathway mediates growth-promoting and anti-apoptotic signaling, while the p38 and JNK pathways play crucial roles in cellular stress responses such as growth arrest and apoptosis. In addition, the p38 and JNK pathways are involved in inflammatory responses. Dysregulation of these critical signal transduction systems is involved in the aetiology of various life-threatening diseases, including cancer, autoimmune diseases, and neurodegenerative disorders.

Since these MAPKs exert their biological effects through the phosphorylation of their substrate proteins, the identification of which is indispensable for comprehensive understanding of regulatory mechanisms of critical biological functions. By developing a novel screening strategy, we have isolated several new MAPK substrates from human cDNA libraries. These substrates include regulatory molecules for the expression of immediate early response genes and for assembly of the actin-cytoskeleton, and several Ser/Thr protein kinases that regulate cell proliferation and apoptosis. We confirmed that these molecules were indeed directly phosphorylated by one (or more) of the human MAPKs *in vitro* as well as *in vivo* in response to mitogenic and/or stress stimuli. Thus, these molecules are bona fide substrates of MAPKs. The biological functions of these novel substrates are under investigation in our laboratory.

3. Regulation of PLK4 activity and centrosome integrity under stress

Takanori Nakamura, Noriko Nishizumi-Tokai, Moe Matsushita, Eriko Mikoshi, Kana Arai, Mayu Sugisaki, and Mutsuhiro Takekawa

Centrosomes serve as the microtubule-organizing centers (MTOCs) and regulate the assembly of mitotic spindles in animal cells. In order for cells to undergo normal bipolar cell division, the single interphase centrosome must duplicate precisely once per cell cycle. The rigorous control of centrosome numbers is critical for accurate chromosome segregation at cell division and for maintenance of the stability of genomes. We have previously demonstrated a direct functional link between the SAPK pathways and Polo-like kinase 4 (PLK4), an evolutionarily conserved main regulator of centrosome duplication. Upon stress stimuli, stress-responsive MAPKKKs directly phosphorylated and activated PLK4. Stress-induced, MAPKKK-mediated, PLK4 activation provides survival signaling and promotes centrosome duplication. At the same time, however, SAPKs and the tumor suppressor p53, both of

which are also activated by various stress stimuli, cooperated to counteract PLK4 activity, thereby preventing centrosome amplification. We demonstrated that simultaneous inactivation of SAPKs and p53 in cells exposed to stress allowed unchecked activation of PLK4, leading to centrosome overduplication and chromosomal instability, both of which are hallmarks of cancer cells. This co-operation between SAPKs and p53 explains why both p53 and the MKK4 MAPKK (a SAPK activator) are frequently mutated simultaneously in cancer, in which centrosome number is often increased after stress.

In this year, we investigated the molecular mechanism as to how PLK4s pecifically localizes to centrosomes. By generating a series of deletion mutants of PLK4, we identified the region of PLK4 that is responsible for its centrosomal localization. Furthermore, we have identified several molecules that selectively interact with the centrosome localization sequence of PLK4 by mass spectrometry analyses. Depletion of some of these PLK4-binding proteins by siRNAs inhibited the centrosomal localization of PLK4. Therefore, these proteins contribute to the subcellular localization of PLK4 and to the maintenance of centrosome integrity. The precise function of these molecules is under investigation in our laboratory.

4. Elucidation of the physiological functions of MCRIP1, an ERK substrate, duringepithelial-to-mesenchymal transition and embryonic development.

Jane S. Weng, Sae Uchida, Takanori Nakamura, Yuji Kubota, and Mutsuhiro Takekawa

Epithelial-to-mesenchymal transition (EMT) is a cellular trans-differentiation program whereby epithelial cells lose their epithelial characteristics and acquire a migratory, mesenchymal phenotype. This phenomenon is involved in a wide range of biological process, including embryonic development, tissue repair, and tissue fibrosis. Furthermore, inappropriate reactivation of the EMT program in malignant epithelial cells is considered to be a major mechanism for the induction of tumor invasion and metastasis. Therefore, comprehensive understanding of the molecular basis of EMT is crucial for the development of novel therapeutic interventions for cancer. Although TGF-β signaling is a prominent mediator of EMT, various other signaling pathways also contribute this process. In particular, it has been shown that hyper-activation of ERK signaling by certain oncogenes, is sufficient to induce EMT in many, if not all, types of cells. Besides ERK signaling, C-terminal binding protein (CtBP), which is a core component of the transcriptional co-repressor complex that contains histone modifying enzymes (e.g., histone deacetylases and methyltransferases), is involved in epigenetic gene silencing of E-cadherin during EMT. However, the functional relationship, if any, between ERK signaling and CtBP remains unclear.

We have identified a novel ERK substrate, designated MCRIP1, which mediates functional crosstalk between ERK signaling and CtBP-mediated gene silencing. MCRIP1 is a previously uncharacterized protein, but is highly conserved in all vertebrates. CtBP is recruited to the promoter elements by interacting with the DNA-binding transcriptional repressor ZEB1. We found that MCRIP1 binds to CtBP, thereby competitively inhibiting CtBP-ZEB1 interaction. However, when phosphorylated by ERK, MCRIP1 dissociates from CtBP, allowing CtBP to interact with ZEB1. In this manner, the CtBP complex is then recruited to, and silences the E-cadherin promoter by inducing histone modifications. Expression of a constitutively-CtBP-binding MCRIP1 mutant profoundly inhibited ERK-induced EMT. These results delineate a molecular mechanism by which ERK signaling induces epigenetic silencing of tumor suppressive genes. The physiological functions of MCRIP1 in the regulation of embryonic development are currently under investigation.

5. MEK mutations associated with congenital diseases and sporadic cancers elicit distinct spatio-temporal properties of ERK signaling.

Yuji Kubota, Yusuke Takagi, Yosuke Kawabe, Ko Fujioka, Yuta Nakano and Mutsuhiro Takekawa

The ERK pathway is frequently hyper-activated by various oncogenes, including receptor tyrosine kinases, Ras, and Raf, inhuman cancers. Interestingly, more than 20 different mutations in the human MEK1/2 genes have recently been identified in sporadic cancers and in congenital Ras/MAPK syndromes. The Ras/MAPK syndromes are genetic diseases that manifest symptoms of facial dysmorphisms, heart defects, mental retardation, and an increased risk of developing cancer. MEK1 mutations have also been identified in melanoma cells that are resistant to the treatment with a B-Raf inhibitor. However, the precise effects of MEK mutations on its enzymatic activity and on carcinogenesis remain elusive.

We investigated the biochemical properties of MEK mutants and found that such mutations rendered MEK constitutively active. We also identified a bunch of genes whose expressions were up-regulated when the ERK pathway is aberrantly activated by the MEK mutants. Importantly, some of these genes are indeed strongly expressed in various human cancer cell lines as well as in clinical cancer tissues, suggesting that these genes are cancer-specific antigens and are thus good targets for

developing novel therapies interventions for cancer.

Publications

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