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 ongoing research projects in our laboratory aim to elucidate the regulatory mechanisms of intracellular signal transduction systems that govern cell-fate decisions, including mitogen-activated protein kinase (MAPK) cascades and stress granules. Dysregulation of these signaling systems is implicated in a wide range of life-threatening diseases, such as cancer, autoimmune disorders, neurodegenerative diseases, and type 2 diabetes. In parallel with basic mechanistic studies, our laboratory also seeks to develop novel diagnostic and therapeutic strategies for currently intractable diseases in which these pathways play critical roles.

1. Analysis of the mechanisms by which living organisms adapt to environmental stress and maintain homeostasis

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Living organisms are constantly exposed to diverse environmental stresses, including ultraviolet and ionizing radiation, oxidative stress, and fluctuations in temperature and osmolarity. These physicochemical stimuli increase in a graded manner, making it challenging for cells to discriminate between harmless fluctuations and damaging conditions. To preserve tissue integrity and organismal homeostasis, cells must ignore weak stress signals while executing decisive responses, such as apoptosis or inflammation, only when stress exceeds a critical threshold.

The c-Jun N-terminal kinase (JNK) signaling pathway plays a central role in cellular stress responses in vertebrates. Previous studies have demonstrated that JNK activation exhibits switch-like behavior: low levels of stress fail to activate JNK, whereas stress above a threshold elicits near-maximal activation without intermediate states. This ultrasensitive response prevents unnecessary biological reactions and ensures binary cell-fate decisions under severe stress conditions. However, the molecular mechanism responsible for converting graded stress stimuli into such switch-like JNK responses has remained poorly understood.

This year, we elucidated a molecular mechanism

underlying this analogue-to-digital conversion in stress-induced JNK signaling. Our work focuses on MKK4, a MAPK kinase that plays a dominant role in JNK activation in response to environmental stress. Unlike other components of MAPK cascades, MKK4 is predominantly localized in the nucleus, despite being phosphorylated and activated in the cytoplasm. The physiological significance of this atypical subcellular localization has long been unclear.

We found that, under unstressed conditions, MKK4 shuttles slowly between the nucleus and cytoplasm. In contrast, environmental stress markedly accelerates this nucleocytoplasmic shuttling through a JNK-dependent feedback mechanism that regulates both nuclear import and export of MKK4. Importantly, this stress-induced increase in shuttling rate, together with the predominant nuclear localization of MKK4, functions as an effective analogue-to-digital converter. Below a critical stress threshold, JNK activation remains suppressed; once the threshold is exceeded, rapid MKK4 shuttling enables robust, switch-like JNK activation. These findings reveal that spatiotemporal regulation of MKK4, rather than changes in kinase activity alone, can generate threshold behavior in cellular stress responses and ensure appropriate cell-fate decisions under fluctuating environmental stress.

2. Regulation and roles of the stress-responsive p38 and JNK MAPKs under stress conditions

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In mammalian cells, diverse extracellular cues, including environmental stressors, are translated into appropriate cellular responses through MAPK signaling pathways. Among these, the p38 and c-Jun N-terminal kinase (JNK) pathways serve as central regulators of cellular stress responses and play critical roles in apoptosis, inflammation, and cell-fate decisions. These pathways are activated via hierarchical kinase cascades consisting of MAPKKKs, MAPKKs, and MAPKs, ultimately leading to the phosphorylation of transcription factors, such as Jun and ATF2, and the induction of stress-responsive gene expression programs.

Although more than a dozen stress-responsive MAPKKKs have been identified in mammalian cells, the functional specificity and physiological roles of individual MAPKKKs remain incompletely understood. We previously identified MTK1 as a stress-responsive MAPKKK and demonstrated that it is selectively activated by members of the GADD45 protein family. Building on this work, this year we systematically investigated the regulation and function of stress-activated protein kinase (SAPK) signaling components by generating and analyzing cell lines deficient in GADD45 proteins, MTK1, and downstream SAPKKs.

Our analyses revealed that the GADD45β–MTK1 axis mediates critical crosstalk between ERK and p38/JNK signaling under oncogenic stress conditions. In normal cells, sustained ERK hyperactivation induces prolonged expression of the transcription factor EGR1, leading to upregulation of GADD45β and subsequent activation of MTK1-dependent p38/JNK signaling. Transcriptome analyses demonstrated that this signaling circuit induces genes involved in apoptosis and immune responses, thereby acting as an intrinsic tumor-suppressive mechanism. In contrast, this pathway is frequently disrupted in cancer cells due to downregulation of EGR1, GADD45β, and/or MTK1, allowing evasion of oncogenic stress-induced apoptosis.

In addition to cell-based studies, we have generated MAPKKK knockout mouse models and are currently analyzing their roles at the organismal level, particularly in chronic inflammatory diseases such as metabolic dysfunction–associated steatohepatitis (MASH) and rheumatoid arthritis.

3. Identification of novel substrates of mitogen-activated protein kinases and their roles in human cancer.

Ryoko Ando, Yuto Ishii, Yuji Kubota, and Mutsuhiro Takekawa

MAPKs are central regulators of intracellular signaling pathways that control cell proliferation, differentiation, and survival. Among them, the ERK pathway transduces mitogenic signals downstream of growth factor receptors and is frequently dysregulated in human cancer. Upon growth factor stimulation, ERK becomes activated through the Ras-Raf-MEK cascade and subsequently phosphorylates a variety of substrate proteins in both the cytoplasm and nucleus, thereby promoting gene expression programs that drive cell growth and tumorigenesis.

Because ERK exerts its biological functions primarily through substrate phosphorylation, systematic identification of ERK target proteins is essential for understanding ERK-dependent regulation in physiological processes and the etiology of human diseases, including cancer. Although several ERK substrates have been characterized, accumulating evidence suggests that many functionally important substrates remain unidentified.

In our laboratory, we have employed complemen-

tary screening approaches to systematically identify novel ERK substrates. These include interaction-based screening using a yeast three-hybrid system, as well as phosphorylation-dependent mobility shift analysis using Phos-tag SDS-PAGE. Through these strategies, we have identified multiple previously uncharacterized ERK substrate proteins, including MCRIP1, NELF-A, and additional candidate ptoteins. Notably, these substrates encompass factors involved in RNA metabolism, transcriptional regulation, and signaling pathways that control cellular growth and survival.

We have demonstrated that these candidate proteins are directly phosphorylated by ERK both in vitro, using purified ERK and recombinant substrates, and in vivo, following mitogenic stimulation of cultured cells. These results establish them as bona fide ERK substrates. Current studies in our group focus on elucidating the biological and pathological consequences of ERK-dependent phosphorylation of these molecules. In particular, we are investigating how dysregulation of these phosphorylation events in cancer cells contributes to tumor progression, metastatic potential, and resistance to anticancer therapies. Furthermore, we are exploring the possibility that targeting the functions or regulatory mechanisms of these newly identified ERK substrates may provide novel therapeutic strategies for cancer and other human diseases.

4. Role of stress granule assembly in regulation of cellular stress response

Sayoko Akiike, Noriko Nishizumi-Tokai, Yuji Kubota, and Mutsuhiro Takekawa

In response to environmental stresses, cells either activate adaptive defense mechanisms to promote survival or initiate cell death signaling, depending on the intensity and nature of stress. One of the major cellular defense mechanisms is the assembly of stress granules (SGs). SGs are cytoplasmic ribonucleoprotein foci that form when eukaryotic cells are exposed to specific stresses, such as ER stress, heat shock, hypoxia 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-oligomerization of certain RBPs such as G3BP. Under various stresses, eIF2α is phosphorylated by distinct stress-sensing kinases. 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 G3BP1/2, 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 RBPs by liquid-liquid phase separation (LLPS) promotes the formation of discrete cytoplasmic foci termed SGs. Although SGs were initially considered to control RNA metabolism and translation reprograming under stress, their roles in these processes remain obscure. In contrast, increasing evidence shows that SGs function as signaling hubs by concentrating several signaling molecules into the granules, and promote adaptive stress responses. However, the precise function of SGs in the regulation of cell-fate decisions under stress remains ill-defined.

In the past year, we have focused on elucidating the molecular mechanisms underlying stress-dependent assembly of stress granules. Through detailed biochemical and cell biological analyses, we identified a previously unrecognized regulatory mechanism that controls SG formation in response to specific stress conditions. Our findings indicate that precise regulation of this mechanism is essential for proper SG assembly and adaptive stress responses. Importantly, we also found that disruption of this SG regulatory pathway leads to aberrant SG dynamics and is associated with cellular phenotypes relevant to neurodegenerative disorders. These observations suggest that failure of appropriate SG assembly may contribute to the pathogenesis of neurodegenerative diseases. We are currently conducting further studies to define the molecular basis of this dysfunction and to clarify its relevance to disease onset and progression.

5. Identification of genes whose expression is controlled by MAPK signaling pathways.

Mitsuka Takahashi, Junichiro Nashimoto, Ryosuke Hiranuma, Yuji Kubota, Noriko Nishizumi-Tokai, and Mutsuhiro Takekawa

Sequential activation of protein kinases within MAPK cascades is an evolutionary-conserved mechanism of intracellular signaling in the eukaryotic world. While ERK is mainly activated by mitogenic stimuli, p38 and JNK are preferentially activated by various environmental stresses. Therefore, p38 and JNK are collectively called 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 pivotal roles in cellular stress responses such as growth arrest and apoptosis. In addition, the p38 and JNK pathways are involved in inflammatory responses. Perturbation of these crucial signal transduction pathways is involved in the pathophysiology of various life-threatening diseases, including cancer, autoimmune diseases, and neurodegenerative disorders.

The initial cellular response to various environmental cues, such as growth factors, environmental stresses, and cytokines, is the transcriptional regula-

tion of a set of genes that control a wide variety of biological functions. MAPK signaling pathways are known to play crucial roles in this process. Previous studies have shown that MAPKs directly phosphorylate and activate a bunch of transcription factors and regulators. For instance, the transcription factor ELK-1, which is a member of the ternary complex factor (TCF) subfamily, is a substrate of ERK. TCFs interact with a second transcription factor, serum response factor (SRF), and these two transcription factors jointly bind and activate serum response elements (SREs) in the promoters of immediately early genes (IEGs). Moreover, upon stress stimulation, p38 and JNK MAPKs directly phosphorylate activating transcription factor 2 (ATF2). ATF2 binds either to CRE response elements as a homodimer, or to both AP-1 and CRE sequences as a heterodimer, in which ATF2 forms a complex with other members of the ATF family or with Jun/Fos family members, thereby inducing target gene expression.

We have comprehensively searched for human genes whose expression is transcriptionally regulated by the MAPK signaling pathways and have succeeded in identifying dozens of such genes. Interestingly, these transcripts include not only protein-coding mRNAs but also various non-coding, functional RNAs. We confirmed that some of these transcripts were indeed expressed preferentially in cancer cells with hyper-ERK activity or in cells treated with certain types of stresses. The roles of these MAPK-dependent transcripts in the regulation of cell fate decisions are currently under investigation in our laboratory.

Publications

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武川睦寛　医学のあゆみ「特集　ストレス応答の分子メカニズム－最新知見と臨床応用への展望－」（武川睦寛編）」293巻7号（2025）