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

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In mammalian cells, extracellular stimuli (e.g., growth factors and environmental stresses) activate specific intracellular signaling pathways that regulate diverse cellular processes, including cell proliferation, survival, and death. The MAPK pathways, which consist of three tiers of sequentially activating protein kinases (i.e., MAPKKK, MAPKK, and MAPK), are key signaling systems that govern cell fate decisions. In mammals, there are at least three distinct MAPK signaling pathways, namely p38, JNK, and ERK pathways. The p38 and JNK pathways preferentially respond to various stresses such as oxidative stress, heat shock, and high osmolality. Upon stress, one or more stress-responsive MAPKKKs are activated, which in turn phosphorylate and activate their cognate MAPKKs, leading to the activation of p38/JNK. Activated p38/JNK then phosphorylate various substrates, including transcription factors (e.g., Jun and ATF2), and modulate their transcriptional activity, thereby regulating gene expression and cellular stress responses (e.g., apoptosis and cytokine production). Notably, sustained activation of p38/JNK promotes apoptosis.

Previous studies have shown that more than a dozen stress-responsive MAPKKKs (e.g., MTK1, ASK1, TAK1, and ZAK) exist in mammalian cells. Although these MAPKKKs can be activated by distinct sets of stress stimuli, their precise roles remain ill-defined. We have previously identified the human stress-responsive MAPKKK, MTK1, and demonstrat-

ed that the GADD45 family proteins (GADD45α/β/γ) specifically activate MTK1. This year, by establishing various cell lines deficient in SAPK signaling molecules (e.g., GADD45, MTK1, SAPKKs, or others), we investigated their regulation and function, and uncovered their roles in carcinogenesis, DNA-damage response, inflammation, and cell growth control. In particular, we found that the GADD45β-MTK1 signaling axis mediates ERK-p38/JNK crosstalk under oncogenic stress conditions and plays a key role in tumor suppression. We demonstrated that, in normal cells, hyperactivation of ERK signaling by oncogenes induces GADD45β expression through the prolonged induction of the TF EGR1, and leads to MTK1-mediated, sustained p38/JNK activation. Transcriptome analyses revealed that this ERK-p38/JNK crosstalk upregulates a set of genes involved in apoptosis and immune response, thereby preventing carcinogenesis. Importantly, ERK-induced GADD45β expression and the resulting MTK1-p38/JNK activation are often abolished in cancer cells due to aberrant downregulation of EGR1, GADD45β, or/and MTK1. Dysregulation of GADD45β–MTK1 signaling impedes oncogenic stress-induced apoptosis in cancer cells, allowing tumor development and progression. Our findings delineate how cells sense and respond to oncogenic stress, and how this mechanism is disrupted in human cancer.

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

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

Mitogen-activated protein kinases (MAPKs) are key regulators of intracellular signaling pathways that orchestrate a wide range of cellular processes, including cell proliferation, differentiation, and survival. Among these, the ERK pathway transduces mitogenic signals and plays a pivotal role in a wide array of biological processes, including cell proliferation, differentiation, and carcinogenesis. Upon stimulation of cells with growth factors such as epidermal growth factor (EGF), their respective receptor tyrosine kinases (RTKs) activate Ras and recruit Raf family kinases to the plasma membrane, which promotes Raf activation. Activated Raf phosphorylates and activates MEK1/2, which in turn activate ERK1/2 by phosphorylation. A portion of the activated ERK then translocates to the nucleus where it phosphorylates and activates specific substrate proteins, including several TFs (e.g., ELK1 and Sp1), and promotes cell growth and tumorigenesis. Since ERK exerts is biological effects through the phosphorylation of its substrate proteins, the characterization of these substrates are crucial for understanding the regulatory mechanisms of critical biological phenomena and the etiology of human cancer. In addition to known ERK substrates (e.g., RSK, ELK1, and Sp1), recent evidence suggests that many unrecognized substrates remain to be discovered. Identifying these proteins can shed light on previously unexplored regulatory networks influencing cell cycle progression, RNA metabolism, and cell death pathways.

In our laboratory, we have employed multiple screening strategies to uncover novel ERK substrate proteins, including a yeast three-hybrid system and a Phos-tag SDS-PAGE analysis, Through these approaches, we have isolated previously uncharacterized substrates of ERK, sucha as MCRIP1, NELF-A, and others. These substrates proteins include signaling molecules involved in RNA metabolism, growth-promoting gene expression, and the regulation of cell fate decisions. We confirmed that each of these candidate substrates is directly phosphorylated by ERK both in vitro, using purified ERK and recombinant substrates, and in vivo, following mitogenic stimulation in cultured cells. Thus, these molecules are bona fide substrates of ERK. Ongoing research in our group focuses on defining the biological and pathological implications of these phosphorylation events. In particular, we are currently examining how altered phosphorylation states of these novel substrates in cancer influence tumor progression, metastatic behavior, and resistance to chemotherapeutic agents. Moreover, we are investigating whether modulating biological activity of these substrates could offer new therapeutic avenues in cancer or other human diseases.

3. Role of stress granule assembly in regulation of cellular stress ad response

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In dealing with environmental stresses, human cells either activate defense mechanisms to survive or initiate cell death signaling, depending on the level and type of stress. One of the major cellular defense mechanisms is the assembly of stress granules (SGs). SGs are cytoplasmic ribonucleoprotein foci that appear when eukaryotic cells are exposed to specific types of stress 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. In cells under various stresses, eIF2α is phosphorylated by several different

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 such as the protection of cells from apoptosis and pyroptosis. However, the precise function of SGs in the regulation of cell-fate decisions under stress remains ill-defined.

Recent work in our laboratory has elucidated new connections between stress SGs, which promote cell survival during stress, and the NLRP3 inflammasome pathway, which orchestrates pyroptosis and inflammation in response to viral or other pathogenic signals. This year, we identified DHX33, a viral RNA sensor for the NLRP3 inflammasome, as a SG component, and the SG-nucleating protein G3BP as an NLRP3 inflammasome component. We also found that a decrease in intracellular potassium (K^+) concentration, a key common step in NLRP3 inflammasome activation, markedly inhibited SG assembly. This discovery suggests a mutually exclusive relationship between SGs and the NLRP3 inflammasome under certain stress conditions: when macrophages are exposed to stress stimuli with the potential to induce both SGs and the NLRP3 inflammasome, such as cytoplasmic poly(I:C) stimulation and viral infection, the cells preferentially form the NLRP3 inflammasome and avoid SG assembly by sequestering G3BP into the inflammasome and by inducing a reduction in intracellular K^+ levels. Thus, under such conditions, DHX33 is primarily utilized as a viral RNA sensor for the inflammasome. Our data illuminate a critical regulatory point in the interplay between cell survival mechanisms (SG formation) and inflammatory cell death (pyroptosis) during viral infection, and delineate a molecular mechanism that regulates cell-fate decisions and anti-viral innate immunity under stress.

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

Shuri Komai, 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 the classical ERK MAPK is mainly activated by mitogenic stimuli, two relatively newly identified MAPKs, p38 and JNK, are preferentially activated by various environmental stresses. Therefore, p38 and JNK MAPKs 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 regulation 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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