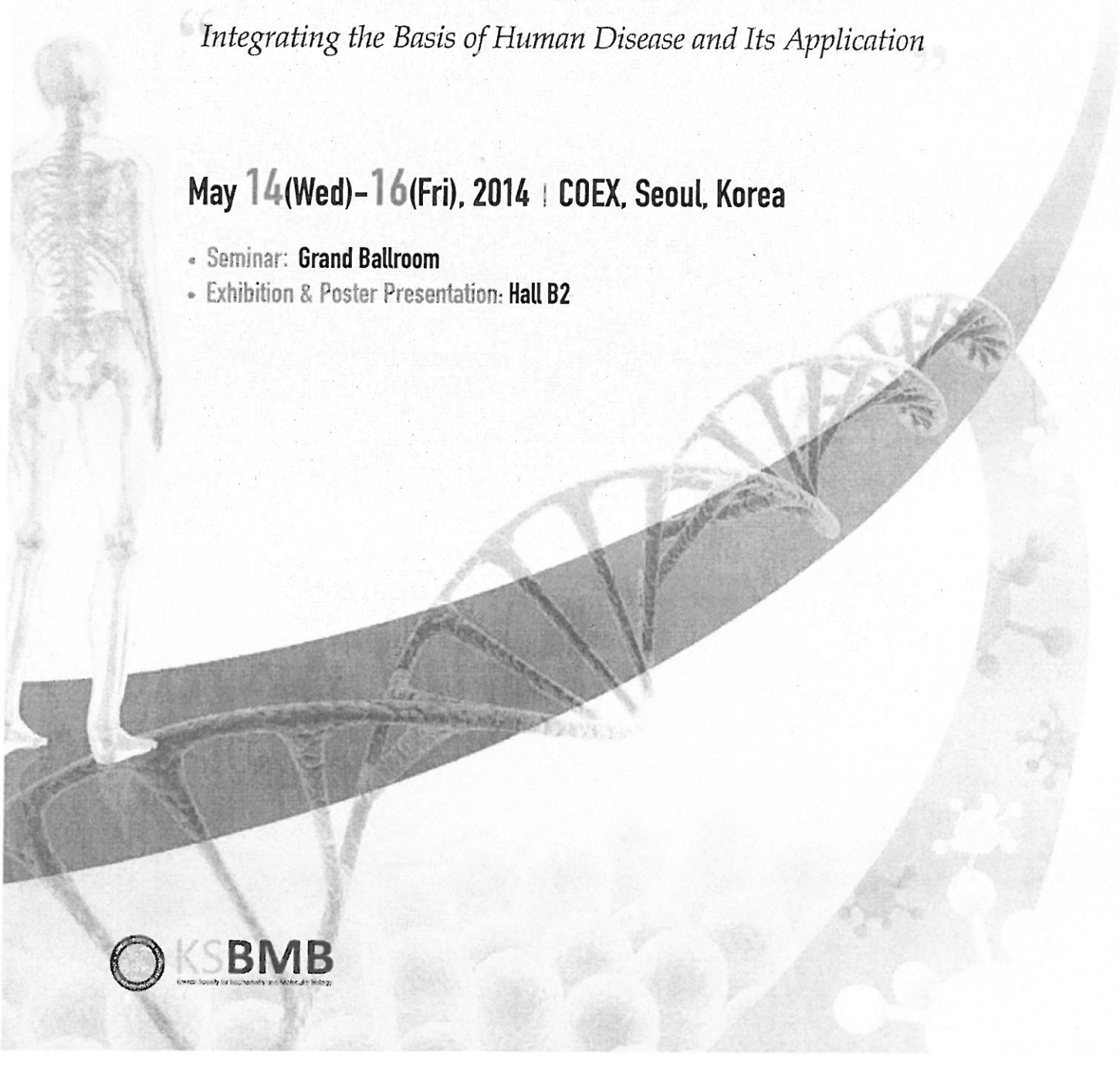
2014 KSBMB Annual Meeting

Integrating the Basis of Human Disease and Its Application

May 14(Wed)-16(Fri), 2014 | COEX, Seoul, Korea

- Seminar: **Grand Ballroom**
- Exhibition & Poster Presentation: **Hall B2**



KSBMB

R-1

Phosphorylation on TRPV4 serine residue 824 enhances its association with PGM1

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The TRPV4 cation channel, a member of the TRP vanilloid subfamily, is expressed in a broad range of tissues and participates in the generation of a Ca2+ signal and/or depolarization of membrane potential. Here, we identified human PGM1, an enzyme that converts glucose-6 phosphate to glucose-1 phosphate in the glycolysis pathway, as the first auxiliary protein of TRPV4 Ca2+ channels using yeast two hybrid system, coimmunoprecipitation, confocal microscopy, and GST pull-down assays. TRPV4 forms a complex with PGM1; this interaction is mediated by the C-terminal cytoplasmic domain of TRPV4. Previously, we demonstrated that TRPV4 serine residue 824 (S824) is phosphorylated by SGK1. Here, we elucidate the effect of TRPV4 S824 phosphorylation on TRPV association with PGM1. An inactivated mutant version of TRPV4, S824A, exhibited a decreased ability to bind PGM1, whereas an activated phosphomimetic mutant version of TRPV4, S824D, exhibited enhanced binding to PGM1. Thus, formation of the TRPV4/PGM1 complex and localization of this complex to the plasma membrane appear to be regulated by the phosphorylation status of residue S824 in TRPV4. This newly identified interactor appears to play a role in the molecular pathways modulating transport activity or glucose metabolism, respectively.

R-2

HBx-induced immune cell recruitment is mediated by chemokine stromal cell-derived factor-1 via endoplasmic reticulum stress

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Hepatitis B virus X protein is a major factor in the HBV-induced disease developments. Stromal cell-derived factor-1 is a small cytokine that is strongly chemotactic for lymphocytes. We explored the role of HBx on recruitment of HBV-induced virus-nonspecific immune cells into liver. Immune cells recruitment and SDF-1 expression level significantly increased in livers of HBx-transgenic mice and X-box binding protein-1 significantly increased SDF-1 gene expression. Finally, we confirmed that immune cell recruitment into liver tissues of HBx-TG mice was diminished by a chemokine receptor antagonist. Therefore, HBx increases ER stress-dependent SDF-1 expression and induces HBV-induced immune cell recruitment into liver.

R-3

The p300 cooperates with c-Jun and PARP-1 at the p300 binding site to activate RhoB transcription in NSC126188-mediated apoptosis

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Here, we present data that the p300 binding site in the RhoB promoter is crucial for the binding of p300 and its partner transcription factors to activate RhoB transcription in NSC126188-mediated apoptosis. NSC126188 increases expression of p300 and c-Jun. Conversely, knockdown of p300 decreases RhoB expression in the presence of NSC126188. Interestingly, poly(ADP-ribose) polymerase-1 (PARP-1) was associated with the p300 binding site. PARP-1 knockdown inhibited NSC126188-mediated RhoB expression. In nuclear extracts of cells treated with NSC126188, p300, PARP-1, and c-Jun interacted and bound the p300-binding site. Furthermore, chromatin immunoprecipitation analysis revealed strong p300 binding and weak c-Jun binding at the p300 binding site in cells treated with NSC126188. It was also shown c-Jun played a crucial role for p300 binding to its binding site. However, PARP-1 did not directly bind the p300 binding site, suggesting a bridging role between p300 and c-Jun. In addition, overexpression of p300, PARP-1, or c-Jun dramatically enhanced RhoB promoter activity when it contained the wild type sequence but not mutated sequences, indicating the crucial role of the p300 binding site for NSC126188-induced transcription of RhoB. Taken together, these data suggest that during NSC126188-mediated apoptosis, p300 is recruited and cooperates with c-Jun and PARP-1 at the p300 binding site to activate RhoB transcription.

R-4

The function of interaction between NBS1 and DDB1 in DNA damage checkpoint

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In eukaryotic cells, a variety of DNA damage checkpoint control mechanisms help to maintain genomic integrity. These regulatory systems scrutinize the genome for the existence of damaged or incompletely replicated DNA. We use Xenopus egg extracts mainly as a model system to study the DNA damage checkpoint. Because DNA damage checkpoint systems are well conserved, we can easily adapt the findings from egg extracts to human cells. NBS1, a member of the Mre11-Rad50-NBS1 (MRN) complex, is an important protein associated with DNA Damage Response (DDR). In this study, we identified DNA damage-binding protein 1 (DDB1) as an interacting partner of NBS1. DDB1 is known as a DDR sensor protein in UV-induced DNA replication block. By using in-vitro pull-down and immunoprecipitation assays in egg extracts and human cell, we found that NBS1 and DDB1 interact, mostly on the N-terminal region of NBS1 and the C-terminal region of DDB1. Also, the interaction is enhanced by damage-dependent manner in the nucleus. These novel findings of the interaction between NBS1 and DDB1 will help to better understand DDR system. The study of this pathway could lead to new approaches to the protection from cancer development, since DDR pathways are the cell's major protectors from cancer development.

R-5

The role of PCNA-NBS1 interaction in DNA damage checkpoint

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DNA damage checkpoint is an important mechanism that controls the integrity of the genomic DNA. When a defect is detected in the DNA, this mechanism allows the cell to be under arrest until repair. Studying this mechanism is meaningful since gaining more knowledge about it may mean a cure for all diseases associated with DNA damge. There are numerous interactions and reactions that take place during this whole process and the interaction between PCNA and NBS1 is the focus of our study. PCNA is a protein known to bind to DNA Polymerase to help the synthesis of DNA leading strand, and NBS1 is a member of the Mre11-Rad50-Nbs1 complex that acts as a double stranded break sensor. We used Xenopus egg extract as the model system for our study, primarily because the DNA damage checkpoint system is well conserved and the extract itself is quite easy to obtain. Through different pull-down assays and immunoprecipitation assays, we found that PCNA and NBS1 show a damage dependent interaction. PCNA shows strong interaction especially at the C-terminus of NBS1. A more detailed study of the mechanism of this interaction will be useful information in further understanding the DNA damage checkpoint and thus, give aid to cancer treatment.

R-7

The role of MST1 in TNFα-mediated NF-κB signaling

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The NF-κB pathway is a central signaling pathway for inflammatory and immune response. Aberrant activation of NF-κB signaling is implicated in multiple disorders such as cancer and autoimmune diseases. The NF-κB signaling events are induced by pro-inflammatory cytokines such as TNFα and IL-1β. TNFα can promote survival, apoptosis, or necrosis, depending on cell type and conditions, through being recognized by TNFR1. In this study, we show that mammalian ste20-like kinase 1 (MST1) regulates TNFα-mediated NF-κB signaling pathway. There was more-prolonged phosphorylation of p65 in MST1-/- MEF than wild-type cells when treated with TNFα. Consistent with this result, depletion of MST1 resulted in more-rapid degradation of IκBα after TNFα treatment. These findings suggest that MST1 negatively regulates TNFα-mediated NF-κB signaling.

The role of MST1 in TNFα-mediated NF-κB signaling

In Young Lee (Master's course) and Eui-Ju Choi

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ABSTRACT

The NF-kB pathway is a central signaling pathway for inflammatory and immune response. Aberrant activation of NF-kB signaling is implicated in multiple disorders such as cancer and autoimmune diseases. The NF-kB signaling events are induced by pro-inflammatory cytokines such as TNFα and IL-1β. TNFα can promote survival, apoptosis, or necrosis, depending on cell type and conditions, through being recognized by TNFR1. In this study, we show that mammalian ste20-like kinase 1 (MST1) regulates TNFα-mediated NF-kB signaling pathway. There was more-prolonged phosphorylation of p65 in MST1$^{-/-}$ MEF than wild-type cells when treated with TNFα. Consistent with this result, depletion of MST1 resulted in more-rapid degradation of IκBα after TNFa treatment. These findings suggest that MST1 negatively regulates TNFα-mediated NF-κB signaling.

PURPOSE

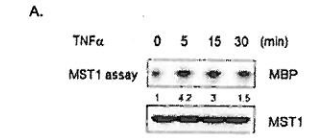
Mammalian Ste20-like kinase 1 (MST1) is a serine/threonine kinase which was initially cloned from a lymphoid cDNA library by PCR in search for homologues of *Saccharomyces cerevisiae* Sterile 20 (Creasy C.L et al. 1995). Sterile 20 is a MAP4K in the yeast mating pathway. MST1 contains a catalytic domain in the amino-terminal region, an autoinhibitory domain in the central region, and a coiled-coil dimerization motif, known as Salvador/ RASSF1/Hippo (SARAH) domain, in the carboxy-terminal region (Scheel H. et al. 2003) The SARAH domain of MST1 mediates self-association as well as heterodimerization with other SARAH-domain containing proteins such as WW45 and Rassf proteins (Scheel H, Hofmann K.2003)

MST1 is ubiquitously expressed and regulates various biological processes including cell proliferation, survival, morphology and motility . It is reported that MST1 is activated by a number of apoptotic or stress stimuli that includes UV radiation, tumor necrosis factor α (TNFα), retinoic acid, serum starvation, heat shock at 55℃, hydrogen peroxide, reactive oxygen species (Kakeya H, Onose R.1998) , and anti-cancer drugs.

While much remains to be discovered, the role of MST1 in immune responses has been partially elucidated. MST1 is associated with regulation of lymphocyte polarity and adhesion, apoptosis of eosinophils, and has implications in somatic hypermutation and class switching in B cells (Ling P,. Lu T.J., Yuan C.J. 2008).

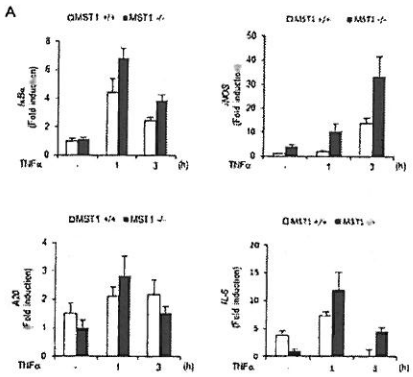
Thus, to gain insight into the biological functions of MST1 in immune responses, We have investigated the possible role of MST1 in TNFα-mediated NF-κB signaling.

Figure 1. MST1 is activated in response to TNFα.



(A) MEF cells were incubated with TNFα (20 ng/ml) for indicated time periods. Cell lysates were subjected to immunoprecipitation with anti-Krs-2 antibody and the resulting precipitates were assessed for MST1 activity by immune complex kinase assay. Lysates were also probed with anti-MST1 antibody.

Figure 3. Deletion of MST1 enhances TNFα-induced NF-κB responsive gene expression.

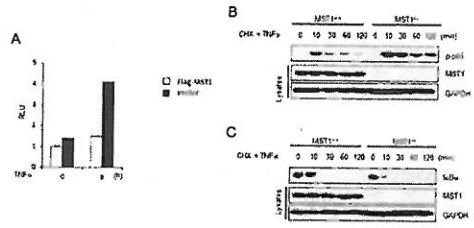


(A) Real-time PCR analysis of the expression of IκBα, iNOS, A20, and IL-6 in MST1+/+ MEF and MST1-/- MEF cells stimulated with TNFα (20 ng/ml) for 1h or 3h.

CONCLUSION

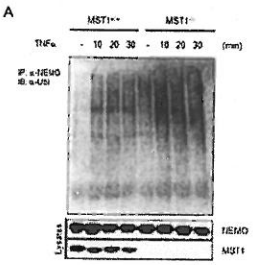
MST1, a member of the STE20 family of kinase, is an upstream activator of MAPK pathways that regulate biological processes such as apoptosis, morphogenesis and cytoskeletal rearrangements. However, the role of MST1 in immune signaling has not been investigated. In this study, we showed supporting evidences that MST1 is a negative regulator of TNFR1-mediated signaling. There was more-prolonged phosphorylation of p65 in MST1$^{-/-}$ MEF than wild-type cells when treated with TNFα. Consistent with this result, depletion of MST1 resulted in more-rapid degradation of IκBα after TNFα treatment. Moreover, deletion of MST1 enhanced the activation of NF-κB and its responsive genes such as IkBa, iNOS, A20 and IL-6. A central regulator for NF-kB activation is the activation of the IKK complex. IKK activation is dependent on phosphorylation by activated TAK1 complex as well as the conjugation of Ub, bound by the IKK subunit NEMO. We observed that the ubiquitination of NEMO was weaker in WT MEFs compared to MST1 KO MEFs, both under basal conditions and upon TNFα treatment. These findings suggest that MST1 functions as a negative regulator of TNFα-induced NF-κB activation.

Figure 2. Effects of MST1 on TNFα-induced NF-κB activation.



(A) NF-κB activities were determined from MST1-/- MEF cells transfected with an NF-κB-luc reporter plasmid and β-galactosidase together with an empty vector or MST1 construct after treatment with TNFα. (B-C) Immunoblot analysis of phosphorylated p65 (B) and the degradation of total IκBα (C) in MST1+/+ MEF and MST1-/- MEF cells simulated for various times (above lanes) with TNFα and cycloheximide (CHX).

Figure 4. Ubiquitination of NEMO is reduced in MST1+/+ MEFs.



(A) MST1+/+ MEF and MST1-/- MEF cells were incubated with TNFα (20 ng/ml) for indicated time periods. Cell lysates were subjected to immunoprecipitation with anti-NEMO antibody and the resulting precipitates were analyzed by immunoblot with anti-ubiquitin. The expression levels of the related proteins were examined by immunoblots with the indicated antibodies.

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