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１.Topic in Research Achievements in the Year 2006

In our current studies, we demonstrate that NLK is involved in the suppression of the Wnt/ -catenin signaling pathway. To gain insights into further unknown function of NLK in the Wnt signaling pathway, yeast two-hybrid screening of a *Xenopus* oocyte cDNA library was carried out using the carboxy-terminus of NLK as bait. One of the positive clones was found to encode a novel *Xenopus* sequence containing a characteristic RING finger domain, and we termed this protein NARF (NLK associated RING finger protein).

Recent data indicate that numerous RING finger proteins can function as E3 ubiquitin-ligases. To determine whether NARF may interact with a partner protein(s) in the ubiquitylation system, we attempted to identify protein(s) that physiologically interact with NARF in mammalian cells, and identified one of the E2 ubiquitin-conjugating enzymes, E2-25K as a NARF-interacting protein. From an *in vitro* ubiquitylation assay, NARF exhibits an auto-ubiquitylating activity in the presence of purified E1 ubiquitin-activating enzyme, E2-25K and ubiquitin, and for this activity the RING finger domain of NARF is indispensable.

To clarify the biological role of NARF, we searched for target proteins that are ubiquitylated by NARF. From the series of assays, we found that NARF could ubiquitylate the transcription factor TCF/LEF, and NLK augmented the ubiquitylating activity of NARF against TCF/LEF by enhancing the association between NARF and TCF/LEF. This enhancement depends on the kinase activity of NLK.

The physiological involvement of NARF in modulating TCCF/LEF protein stability via ubiquitin-proteasome pathway was confirmed using siRNA for NARF. When endogenous NARF expression in 293 cells was suppressed by NARF siRNA, a significant accumulation of endogenous TCF4 was observed and proteasome inhibitor treatment further enhanced the stability of TCF4. We examined the effect of NARF siRNA on the expression of Dickkopf-1 (DKK-1), a target gene of -catenin/TCF and an endogenous Wnt antagonist. Wnt-3a treatment induced the expression of the DKK-1 gene in HeLaS3 cells. When endogenous NARF expression was suppressed with NARF siRNA (siNARF), Wnt-dependent expression of DKK-1 mRNA was further enhanced as compared with the control (siControl) at all examined time points.

Collectively, our findings provide the first evidence that NARF functions in the specific ubiquitylation and degradation of TCF/LEF *in vivo* to suppress the Wnt- -catenin signaling. Here we propose the novel model for regulating the Wnt- -catenin signaling via post-translational modification of TCF/LEF by coordinate interaction between NLK and NARF.

２. Publications in the year 2006

Honma, M., Higuchi, O., Shirakata, M., Yasuda, T., Shibuya, H., Iemura, S., Natsume, T. and Yamanashi, Y. (2006). Dok-3 sequesters Grb2 and inhibits the Ras-Erk pathway downstream of protein-tyrosine kinases. **Genes Cells** 11, 143-151.

○ Yamada, M., Ohnishi, J., Ohkawara, B., Iemura, S., Satoh, K., Hyodo-Miura, J., Kawachi, K., Natsume, T. and Shibuya, H. (2006). NARF, an Nemo-like kinase (NLK)-associated Ring finger protein regulates the ubiquitylation and degradation of T cell factor/Lymphoid enhancer factor (TCF/LEF). **J. Biol. Chem.** 281, 20749-20760.

3 . Abstracts in the year 2006

Hisamoto N, Urushiyama S, Moriguchi T, Ito K, Mitani S, Shibuya H, Matsumoto K. WNK and SPAK regulate excretory organ morphogenesis in C. elegans. 20th IUBMB international congress of biochemistry and molecular biology and 11th FAOBMB congress, Poster session, 2006.