Molecular Cell, Volume *31*

Supplemental Data

TRAF6 Mediates Smad-Independent Activation of JNK and p38 by TGF-β

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Supplemental Experimental Procedures

Plasmids

Expression plasmids for C-terminal Myc-tagged TβRII, TβRI, kinase deficient TβRI (KR), FLAG-tagged constitutively active TβRI (TD), N-terminal FLAG-tagged TRAF2, TRAF6, dominant negative TRAF2 (87-501), TRAF6 (289-522), HA–tagged JNK or ubiquitin, and HA-tagged ubiquitin mutant s were described previously (Feng et al., 1995; Cao et al., 1996; Hsu et al., 1996; Yu et al., 2002, Vong et al., 2005). C-terminal Myc-tagged TβRI (TD), and Smad-binding mutants, TβRImL45 and TβRImL45 (TD), were constructed by subcloning EcoRI and SalI fragments of coding sequence from C-terminal FLAG-tagged TβRI (TD), TβRImL45, TβRImL45 (TD) (Yu et al., 2002) into the pRK5Myc vector (Feng and Derynck., 1995). C-terminal His6 tagged TβRII was constructed by replacing Myc tag with a His6 tag between SalI/HindIII sites of pRK5-TβRII-Myc plasmid. FLAG-tagged p38 were generated by PCR of coding sequence of p38α and inserted into BamHI and SalI sites of N-terminal FLAG-tagged pRK5 vector. PCR-based approaches were also used to generate TRAF6R/Zn (1-288), TRAF6C (358-522), and TRAF6 (C70A).

siRNAs

All siRNAs used in the manuscript were synthesized by Qiagen. The target sequence of the Smad3 siRNA is common to both human and mouse gene has been described (Yang et al., 2006). The target sequences for two mouse TRAF6 siRNAs and one human TRAF6 siRNA are: 5′-CAGGCCTTTACAGCTTCTC-3′ (Mm_TRAF6_1), 5′-GGCCATCACCACGCAGAAC-3′ (Mm_TRAF6_4), and 5′-CAGCGCTGTGCAAACTATATA-3′ (Hs_TRAF6_4), respectively. The target sequence for the nonsilencing control siRNA (NS) is: 5′-AATTCTCCGAACGTGTCACGT-3′.

Supplemental References

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Hsu, H., Shu, H.B., Pan, M.G. and Goeddel, D.V. (1996). TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. Cell *84*, 299-308.

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Figure S1: Deletion mapping of the TβRII binding domain in TRAF6. HEK293 cells were transfected with expression plasmids for Flag-tagged TRAF6 and Myc-tagged TβRII as marked. Cell lysates were subjected to anti-Myc immunoprecipitation followed by anti-Flag immunoblotting. A schematic representation of various deletion constructs of Flag-TRAF6 was shown in Figure 2B.

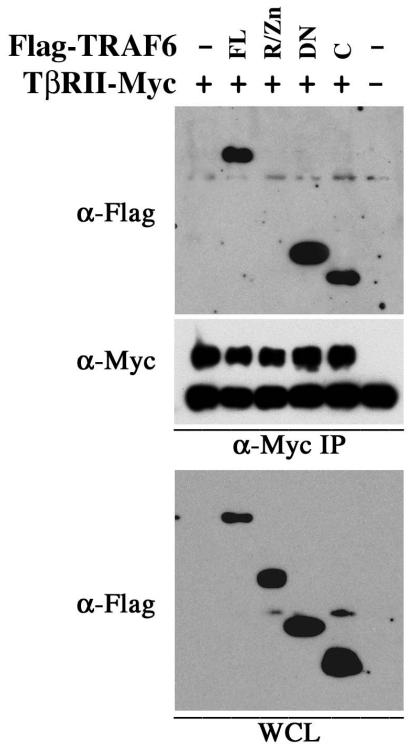

Figure S2: Effect of XIAP RNAi on the activation of JNK and p38 by TGF-β. AML12 cells were treated with TGF-β for 20 min at two days after siRNA transfection. The levels of phospho- or total JNK and p38 in cell lysates were analyzed by western blots.