# Molecular mechanisms of novel regulators in cytokine signal transduction

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## Summary

In a multicellular organism, each cell is exposed to and determined by a combination of different signals during development. An individual signal molecule can exert different effects on different target cells, depending on the composition of the signaling machinery and networks on the outside and inside of the cell. Also, the response of a particular cell to a signal can depend on its environment. The response of a cell to a signal molecule depends on how the cell translates the information. A large number of molecules/regulators in a cell can potentially influence the integration and interpretation of signals. Thus, the identification and characterization of novel regulators is crucial to understand the mechanisms by which signaling pathways and networks function and interact, and help to understand the role of these signaling pathways in cancer and other diseases.

In the studies described in this thesis, we aimed to identify and investigate novel regulators for three major signaling pathways: the TGF-β/BMP, IL-1β/LPS and Wnt pathways (*chapter 1*). To pinpoint novel regulators for each signaling pathway, we utilized different screening methods. In *chapter 2*, we utilized a whole proteomic protein-protein interaction screen and identified UBE2O as a new regulator of BMP signaling. We found UBE2O to monoubiquitinate the negative BMP regulator SMAD6 at lysine 174 and to counteract the inhibitory effect of SMAD6 on BMP7-induced signaling. Ubiquitinated SMAD6 has less binding efficiency for the BMP type I receptor, resulting in reduced inhibition of BMP7-induced adipocyte differentiation. We also found that cysteine 885 in the E2 active site of UBE2O is essential for the monoubiquitination of SMAD6, and that UBE2O could potentiate BMP7-induced adipogenesis.

IL-1β and LPS trigger transcription factor NF-κB activation via the adapter protein TRAF6, a member of the TNF receptor-associated factors and a key signaling protein in the control of innate and adaptive immunity. We uncovered two new regulators of TRAF6 signaling (*chapter 3* and *chapter 4*). Both the E2 enzyme UBE2O and the deubiquitinase USP4 were found to negatively regulate TRAF6-mediated NF-κB and transcription factor AP-1 activation. Misexpression of UBE20 and USP4 affected IL-1β/LPS-induced phosphorylation of key downstream signaling mediators and expression of target genes. These two regulators both were found to target polyubiquitinated TRAF6, but the mechanisms by which they inhibit TRAF6 are different. UBE2O inhibits the formation of polyubiquitin chains of TRAF6 independent of its E2 ligase activity. UBE2O interrupts the interaction of MyD88 and TRAF6, resulting in less ubiquitination of TRAF6 by the E2 complex Ubc13/Uev1a (*chapter 3*). In contrast, the deubiquitinase USP4 binds to and cleaves the polyubiquitin chains of TRAF6. This effect depends on the catalytic site of USP4 (*chapter 4*).

The Wnt pathway is crucial for many cellular processes from embryonic development to adult homeostasis. Our studies were aimed to find whether there is a co-receptor for Wnt signaling besides LRP5/6 (*chapter 5*). In a siRNA screen for membrane

proteins depletion of the related LRP8 showed a strong inhibition on Wnt3a-induced signaling. Further experiments demonstrated that LRP8 interacts with and thereby inactivates the negative Wnt regulator Axin, which results in the nuclear accumulation of the transcriptional co-factor β-catenin. In line with this, functional assays showed a strong positive effect of LRP8 on Wnt3a-mediated osteoblast differentiation and bone formation.

In conclusion, these studies on novel regulators of TGF-β/BMP, IL-1R/TLR4 and Wnt signaling help us to understand how TGF-β/BMP, IL-1R/TLR4 and Wnt control cellular functions such as differentiation, inflammation and apoptosis, which might be relevant for cancer and other diseases.