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## TGF- $\beta$ signaling dynamics in epithelial-mesenchymal plasticity of cancer cells

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# **Appendix**

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**Nederlandse Samenvatting**  
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**Acknowledgements**



## English Summary

In cancer cells, malfunction of transforming growth factor (TGF)- $\beta$  signaling can promote migration and metastasis, in part through the induction of epithelial-mesenchymal transition (EMT). Although strategies targeting TGF- $\beta$  signaling are being explored in clinical trials, the on-target side effects caused by long-term systemic TGF- $\beta$  signaling inhibition limit the clinical approval of TGF- $\beta$  targeted therapies in cancer patients. Therefore, unraveling the regulatory mechanisms of TGF- $\beta$  signaling in cancer (and normal) cells may offer new opportunities to treat cancer patients.

Long non-coding RNAs (lncRNAs) are a class of transcripts without coding potential but some were found to have a pivotal role in regulating signal transduction pathways through various mechanisms. In **Chapter 2**, we performed transcriptomic profiling to screen for TGF- $\beta$ -induced lncRNAs in breast cancer cells. Follow-up loss-of-function studies identified lncRNA Induced by TGF- $\beta$  and Antagonizes TGF- $\beta$  Signaling 1 (*LITATS1*) as a protector of epithelial cells to suppress TGF- $\beta$ -induced EMT and invasive abilities of cancer cells. Mechanistically, *LITATS1* serves as a scaffold to enforce the interaction between TGF- $\beta$  type I receptor (T $\beta$ RI) and the SMAD specific E3 ubiquitin ligase 2 (SMURF2), leading to the increase of polyubiquitination and proteasomal degradation of T $\beta$ RI. *LITATS1* can also sequester SMURF2 protein in the cytoplasm, thereby promoting its export from the nucleus. Analysis of patient samples showed that *LITATS1* expression correlates with a favorable survival outcome in breast and non-small cell lung cancer patients, highlighting the potential of *LITATS1* as a promising prognostic marker. Of note, reintroducing *LITATS1* into highly aggressive breast cancer cells mitigated their migration and extravasation, suggesting that *LITATS1* may be a therapeutic anti-cancer agent.

lncRNAs that activate TGF- $\beta$  signaling in cancer cells may be explored as alternative therapeutic targets to selectively inhibit TGF- $\beta$  signaling and TGF- $\beta$ -induced EMT in cancer cells. In **Chapter 3**, we described how lncRNA Enforcing TGF- $\beta$  Signaling 1 (*LETS1*) promotes TGF- $\beta$ -induced EMT and cancer cell migration by transcriptionally activating a T $\beta$ RI-stabilizing mechanism. In this study, we demonstrated that TGF- $\beta$ /SMAD-induced nuclear *LETS1* interacted with nuclear factor of activated T cells (NFAT5) to facilitate the transcription of orphan nuclear hormone receptor *NR4A1*. NR4A1 alleviates T $\beta$ RI polyubiquitination and potentiates T $\beta$ RI stability by facilitating inhibitory (I)-SMAD7 protein degradation, leading to an activation of TGF- $\beta$ /SMAD signaling, TGF- $\beta$ -induced EMT, and cancer cell migration and extravasation. Thus, we unraveled a novel mechanism by which TGF- $\beta$ /SMAD signaling is fine-tuned at the receptor level through an unannotated lncRNA *LETS1*.

Ovo-like transcriptional repressor 1 (OVOL1) is a vital determinant of epithelial lineage and stimulator of mesenchymal-epithelial transition (MET). However, its interplay with TGF- $\beta$  and bone morphogenetic protein (BMP) signaling is unclear. **Chapter 4** presents that BMP strongly induces the expression of OVOL1, which potentiates BMP signaling in turn. This positive feedback loop is achieved by OVOL1-mediated suppression of TGF- $\beta$ /SMAD signaling. OVOL1 binds to inhibitory SMAD7 and displaces interaction with E3 ligases targeting SMAD7. OVOL1 thereby prevents the polyubiquitination and proteasomal degradation of SMAD7. As a consequence, T $\beta$ RI is destabilized by OVOL1, resulting in the attenuation of TGF- $\beta$  signaling and TGF- $\beta$ -induced EMT, migration in breast cancer cells. In addition, we identified 6-formylindolo(3,2-b)carbazole (FICZ) as a small-molecule compound that can stimulate OVOL1 expression and thereby antagonize (at least in part) TGF- $\beta$ -triggered EMT

and migration in breast cancer cells. Hence, we uncovered a mechanism by which OVOL1 interplays with TGF- $\beta$  and BMP signaling and maintains breast cancer cell epithelial identity.

Taken together, we identified several novel modulators of TGF- $\beta$ /SMAD signaling. We studied the role of these modulators in TGF- $\beta$ -induced EMT and migration in breast and lung cancer cells, and elucidated the mechanisms by which they fine-tune TGF- $\beta$ /SMAD signaling transduction. These studies contribute to a better understanding of the regulatory networks of TGF- $\beta$  signaling and may offer new therapeutic potentials to target TGF- $\beta$  signaling in patients with breast or lung cancer.