TGF-β signaling dynamics in epithelial-mesenchymal plasticity of cancer cells

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Appendix

English Summary

In cancer cells, malfunction of transforming growth factor (TGF)-β signaling can promote migration and metastasis, in part through the induction of epithelial-mesenchymal transition (EMT). Although strategies targeting TGF-β signaling are being explored in clinical trials, the on-target side effects caused by long-term systemic TGF-β signaling inhibition limit the clinical approval of TGF-β targeted therapies in cancer patients. Therefore, unraveling the regulatory mechanisms of TGF-β 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-β-induced lncRNAs in breast cancer cells. Follow-up loss-of-function studies identified **L**ncRNA **I**nduced by **T**GF-β and **A**ntagonizes **T**GF-β **S**ignaling **1** (*LITATS1*) as a protector of epithelial cells to suppress TGF-β-induced EMT and invasive abilities of cancer cells. Mechanistically, *LITATS1* serves as a scaffold to enforce the interaction between TGF-β type I receptor (TβRI) and the SMAD specific E3 ubiquitin ligase 2 (SMURF2), leading to the increase of polyubiquitination and proteasomal degradation of TβRI. *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-β signaling in cancer cells may be explored as alternative therapeutic targets to selectively inhibit TGF-β signaling and TGF-β-induced EMT in cancer cells. In **Chapter 3**, we described how **L**ncRNA **E**nforcing **T**GF-β **S**ignaling **1** (*LETS1*) promotes TGF-β-induced EMT and cancer cell migration by transcriptionally activating a TβR1-stabilizing mechanism. In this study, we demonstrated that TGF-β/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 TβRI polyubiquitination and potentiates TβRI stability by facilitating inhibitory (I)-SMAD7 protein degradation, leading to an activation of TGF-β/SMAD signaling, TGF-β-induced EMT, and cancer cell migration and extravasation. Thus, we unraveled a novel mechanism by which TGF-β/SMAD signaling is fine-tuned at the receptor level through an unannotated lncRNA *LETS1*.

Ovo-like transcriptional repressor 1 (OVOL1) is a vital determinator of epithelial lineage and stimulator of mesenchymal-epithelial transition (MET). However, its interplay with TGF-β 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-β/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, TβRI is destabilized by OVOL1, resulting in the attenuation of TGF-β signaling and TGF-β-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-β-triggered EMT

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

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