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Modulation of Ursodeoxycholyl Lysophosphatidylethanolamide on Pro-fibrogenic Integrin, TGFβ and EGFR Signaling Pathways

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Although it is acknowledged that integrins, TGFβ, and EGFR signalings play an important role in liver fibrosis, the medicine development to inhibit these signaling pathways has been disappointing. Data in this thesis have shed some light on the mechanisms of UDCA-LPE as a potent anti-fibrosis drug.

In order to promote pro-fibrogenic signalings upon ECM binding, integrins recruit focal adhesion kinase (FAK) and SRC which are phosphorylated in response to integrin engagement. Integrins recognize a tripeptide motif composing of L-arginine, glycine, and L-aspartic acid (RGD) present in ECM. In this thesis, it has been shown that UDCA-LPE was able to stimulate an early and transient integrin-dependent phosphorylation of EGFR, c-Raf and ERK in a similar manner to UDCA, TUDCA, and RGD peptide. On the other hand, UDCA-LPE was also able to stimulate a transient activation of PKA and b-Raf in a similar manner to LPE suggesting that UDCA-LPE was able to bind to and at the same time stimulate receptors with both of its UDCA- and LPE-moiety. Immunoprecipitation experiment showed that UDCA-LPE was able to induce the interaction between integrin β1 and LPAR1. Incubation of CL48 liver cells and primary human hepatic stellate cells with UDCA-LPE was followed by a translocation of integrin α2, α3, α5, αv, β1, β3, β4, β5 and β6, but not integrin α1, to the ER and the nuclear envelope. This led to a loss of co-localization of integrins with SRC resulting in dephosphorylation of FAK (Tyr 576/577 and Tyr 925) and SRC (Tyr 416). The translocation of integin β1 was not induced by treatment with UDCA and/or LPE. RGD-containing peptide GRGDSP or LPAR antagonist Ki16425 was able to inhibit UDCA-LPE-induced translocation of integrins. Furthermore, lipid fractionation showed the consistency of the translocation of integrins with UDCA-LPE (from

UDCA-localized fractions to LPE-localized fractions), suggesting that the translocation of UDCA-LPE with its binding integrins was driven by LPE transporters.
The occupation of ligands (including UDCA-LPE and RGD peptide) on integrins was found to induce a selective phosphorylation of TGFβR2 at Ser225, but not at Tyr336, whereas TGFβ1 stimulated phosphorylations of TGFβR2 at both sites. The phosphorylation of TGFβR2 (Ser225) induced a transient phosphorylation of Smad2 (Ser465/467) and Smad3 (Ser423/425). UDCA-LPE further led to the lipid-raft-mediated translocation of TGFβ receptors (TGFβR1 and TGFβR2) resulting in degradation of TGFβR1 and inhibition of Smad2/3 phosphorylation. Experiments by using the blocking GRGDSP peptide or blocking antibodies against integrins indicate that UDCA-LPE-induced translocation of TGFβ receptors and inhibition of Smad2/3 phosphorylation were indeed integrin-dependent. LPAR antagonist Ki16425 was able to reverse UDCA-LPE-induced inhibition of Smad2/3 phosphorylation, suggesting this inhibition was due to LPE-transporter-mediated translocation of TGFβ receptors.
UDCA-LPE induced the internalization of EGFR with a similar mechanism to that of TGFβ receptors. The internalization of EGFR led to EGFR degradation and an inhibition of EGF-induced phosphorylation of FAK (Tyr925) and SRC (Tyr416). Fibronectin or Ki16425 was able to inhibit UDCA-LPE-induced degradation of EGFR suggesting that the degradation was caused by integrin-dependent translocation of EGFR.
Conclusions: UDCA-LPE mediated the translocation of integrins, TGFβ receptors and EGFR leading to a loss of co-localization with their down-stream signaling proteins FAK, SRC and Smad2/3. By inhibiting the crucial pro-fibrogenic signaling pathways, UDCA-LPE emerges as a promising experimental drug candidate for the treatment of liver fibrosis.