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Background: Cholangiocarcinoma (CCA) is a devastating cancer with few effective treatment options. We have previously discovered LCK can activate AXL, a TAM receptor tyrosine kinase (RTK), by phosphorylation of AXL-Y866 (*J Hepatol* 2022). AXL is known to confer therapeutic resistance to solid cancers. However, the role of AXL in CCA, and the impact of AXL-Y866 activation by LCK still remains to be elucidated. Here, we investigated the significance of AXL expression, signaling, and activation by LCK on Y866. **Methods:** We first evaluated the expression levels of AXL in CCA and its association with patient outcome using The Cancer Genome Atlas (TCGA) dataset. Next, to evaluate whether AXL inactivation sensitizes CCA cells to gemcitabine and cisplatin (GC), *AXL* downregulation was achieved via siRNA approach and the selective AXL inhibitor bemcentinib (Bem) using human CCA cell lines, HuCCT1 and RBE, and murine SB-1. We examined 50% inhibitory concentration (IC_{50}) value on GC with or without *AXL* knockdown (KD) using cell viability assay. Then we assessed the efficacy of the combination of GC and Bem utilizing Calcusyn software and apoptosis by Annexin V assay. *In vivo* efficacy was assessed using both a patient derived CCA xenograft and SB-1 a syngeneic model of CCA treated with vehicle, GC, Bem, or the combination. Next, we defined the interactome of wildtype AXL-Y866 compared to AXL-Y866F using biotin proximity labelling methods (BioID), validation of downstream target proteins using Western blot (WB), and a functional study with *AXL*$^{-/-}$ cells reconstituted with either *AXL* WT, or *AXL* Y866F. **Results:** In TCGA cohorts, *AXL* transcripts are more abundant than in normal adjacent liver, and higher levels are correlated with worse clinical outcome. In *in vitro* study, IC_{50} values of GC decreased after *AXL* KD. Synergistic effects were observed in the combination. The combination caused increased apoptosis compared to other treatments. In the *in vivo* studies, the combination suppressed tumor growth and the Ki67 levels, and the phosphorylation levels of PEAK1 decreased in the combination group compared to other groups. In BioID experiments, among the 222 proteins which were detected in both AXL WT and AXL Y866F interactomes, PEAK1 kinase, which localizes to actin cytoskeleton and focal adhesions, was most altered when Y866 was inactivated by the Y866F mutation. In gene ontology analysis, cell-cell adhesion and focal adhesion related proteins were enriched. In WB, we validated that the phosphorylation of PEAK1 decreased after *AXL* or *LCK* knockout (KO) and after Bem treatment in above *in vivo* study. In cell viability assay, reconstitution of *AXL* KO cells with WT *AXL* increased the IC50 to GC while reconstitution with *AXL*-Y866F did not. **Conclusions:** AXL inhibition sensitizes CCA cells to cytotoxic chemotherapy in preclinical model. LCK-AXL Y866-PEAK1 signaling axis is a potential target for the treatment of CCA. Research Sponsor: U.S. National Institutes of Health.