Supplementary figure 1. Overview of the study

Exploration analysis

Dysregulation and prognostic value of estrogen response in CCA tissues in 3 cohorts: GSE89749 cohort, E-MTAB-6389 cohort, TCGA-CHOL and GSE76297 cohort.

Identification of an estrogen response-related genes (ESRGs) utilizing WGCNA algorithm.

Consensus clustering of ESRGs in 118 CCA samples in the GSE89749 cohort.

ES cluster A
ES cluster B

Discovery analysis

Clinical and transcriptomic characteristics of estrogen response in the GSE89749 cohort

Potential cause and spatiotemporal specificity of differential estrogen response

The immune microenvironment differs between different ES clusters.

ES response Characterization

ES cluster A
ES cluster B

ESRS

Clinical significance analysis

Establishment and validation of the ESRS in CCA patients

Potential therapeutic targets and applicable drugs according to ESRS or estrogen response.

ES response-related score (ESRS)

nomogram

Estrogen metabolism genes

ES score in different cell types

ES score within CCA cells

Immune cell infiltration

Complement activation

Prognosis

Anatomy

Clinical stage

Fluke infection

Other CCA clusters

Biological processes

Potential targets: GSEA

Applicable drugs: CMap database

Drug resistance: GDSC database
Supplementary figure 2. Estrogen response predicts poor prognosis in CHOL and differs among different cancer types.

A-E, Kaplan–Meier curves for overall survival of the patients with low or high GSVa score of "HALLMARK_ESTROGEN_RESPONSE_LATE" or "HALLMARK_ESTROGEN_RESPONSE_EARLY" in the GSE89749 cohort, E-MTAB-6389 cohort or TCGA-CHOL cohort. F, TSNE plot according to the expression of genes in "HALLMARK_ESTROGEN_RESPONSE_LATE" for all the 32 types of cancerous tissues in TCGA cohort. G, Forest plot showing the prognostic value of GSVa score of "HALLMARK_ESTROGEN_RESPONSE_LATE" in 15 types of solid tumor.
Supplementary figure 3. Clinical and transcriptomic characteristics of ES clusters in GSE89749 cohort.

A, Optical clustering chosen by delta area. B, Boxplot showing the GSVA score of “HALLMARK_ESTROGEN_RESPONSE_LATE” in different fluke infection status, sex, age, anatomy, histology, T stage, N stage, M stage, clinical stage, CCA cluster and ES cluster. C, Boxplot showing the difference of estrogen response in inflammation and proliferation subtypes in GSE33327 cohort. D, The top biological processes activated or suppressed in ES cluster B versus ES cluster A generated by GSEA. E and F, GSEA plots exhibit the enrichment of metabolism of TCA cycle and amino acid in ES cluster B.
Supplementary figure 4. Estrogen response and the expression of KRT19, ANXA4, COMT, HSD17B1 in single cell level.

A and B, The strength of “HALLMARK_ESTROGEN_RESPONSE_LATE” was evaluated by AUCCell method in single cell level in all cell types (A) and subtypes of CCA cells (B), represented by the colour of the dots. C and D, The expression of COMT, HSD17B1 in all the cells of CCA tissues. E and F, The expression of KRT19 and ANXA4 in CCA cells of different subtypes. G and H, The scatter plots showing the correlation between AUCCell score of “HALLMARK_ESTROGEN_RESPONSE_LATE” and the expression of KRT19 or ANXA4 in cholangiocarcinoma cells. The colour of the dots represent the subtypes of CCA cells. I and J, The expression of KRT19 and ANXA4 in cell trajectory plot.
Supplementary figure 5. Immune cell infiltration and complement activation status in cholangiocarcinoma.

A. Differential infiltration of immune cells in ES cluster A and B in GSE89749 cohort estimated by cibersort. B. Annotation of single cell types in 5 CCA tissues. The cholangiocarcinoma cells were further divided into KRT19+ and ANXA4+ malignancy according to the expression or KRT19 and ANXA4. C. The strength of “GO_COMPLEMENT_ACTIVATION” was evaluated by AUCell method and exhibited in the TSNE plot by colour. D. Ridge plot showing the AUCell score of “GO_COMPLEMENT_ACTIVATION” in all cell types in different cell types of CA tissues. E. The GSEA enrichment plot showing the enrichment of “GO_COMPLEMENT_ACTIVATION” in CCA tumor tissues compared with CCA non-tumor tissues. F. Boxplot showing the expression of complement gene expression in CCA tumor tissues and CCA non-tumor tissues. G-H, Kaplan–Meier curves for overall survival of the patients with low or high expression of C3 and C5 in the GSE89749 cohort.
Supplementary figure 6. Potential therapeutic targets and drugs should not be adopted according to ES response.

A and C, GSEA indicated that “REACTOME_KERATINIZATION” was top enriched pathway in ESRS_high group and “KEGG_DRUG_METABOLISM_CYTOCHROME_P450” was top enriched pathway in ESRS_low group. B and D, Two protein interaction networks were generated out of differential genes in “REACTOME_KERATINIZATION” (B) and “KEGG_DRUG_METABOLISM_CYTOCHROME_P450” (D). The size of the dot represents the fold change of the genes between ESRS_high and ESRS_low groups. E The differentially expressed genes were submitted to CMap mode-of-action (MoA) analysis and the results showed 42 mechanisms of action shared by top 50 compounds which show similar impact on cancer cells to ESRS which should be dismissed for ESRS_high patients.