Therapeutic potential of targeting protein hyper-SUMOylation in cholangiocarcinoma

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BACKGROUND

- **Cholangiocarcinoma (CCA)** comprises a heterogeneous group of malignant tumors with dismal prognosis.
- Given the complex biology of CCA, it is important to identify key molecular mechanisms involved in cholangiocarcinogenesis.
- **Post-translational modifications (PTMs)** provide a rapid mechanism for the activation or inhibition of signaling pathways and metabolism of proteins.
- Alterations in PTMs, including SUMOylation, result in abnormal protein dynamics, cell disturbances and disease.
- **ML792**, a selective SAE1 inhibitor, and **S-adenosylmethionine (SAMe)**, which targets UBC9, are inhibitors of the SUMOylation pathway.

AIM

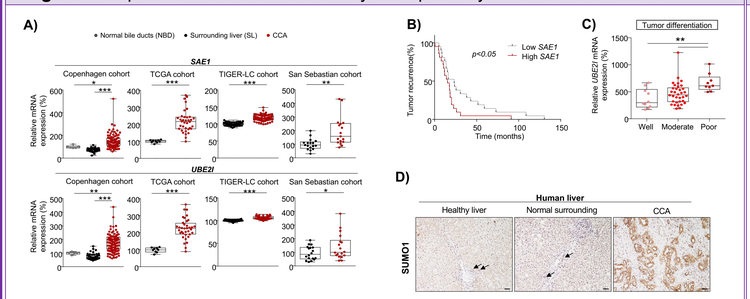
Explore in detail the role of protein SUMOylation in cholangiocarcinogenesis and evaluate its therapeutic potential in experimental models of CCA.

METHODS

- **Expression analysis (mRNA)** of the SUMOylation pathway components (*SAE1*, *UBE2I* and *SUMO1*) in CCA and surrounding liver tissue samples of four independent cohorts: Copenhagen (microarray), TCGA (RNA-seq), TIGER-LC (microarray) and San Sebastian (qPCR).
- **Expression analysis (mRNA and protein)** of SUMOylation in CCA cell lines (EGI1, HUCCT1, TFK1 and WITT) and normal human cholangiocytes (NHC).
- **Identification of SUMOylation targets** by **immunoprecipitation** of SUMO1-conjugated proteins from CCA cell lines and NHC and comparative shotgun **proteomic analyses** by mass spectrometry (MS).
- **Evaluation of the effect of** pharmacologically inhibiting SUMOylation with **SAMe** or **ML792** in CCA cell proliferation, colony formation and survival ***in vitro***.
- **Evaluation of the effect of SAMe** and **ML792** in subcutaneous **mouse models** of CCA.
- **Molecular targeting of SUMOylation** using the **CRISPR/Cas9** methodology in CCA cells.
- Determination of the **impact of CRISPR/Cas9-*UBE2I*** in CCA cell proliferation, colony formation and tumorigenesis.
- Assessment of the **role of SUMOylation** in the **crosstalk** between **CCA cells and cancer-associated fibroblasts** (CAFs), **endothelial cells** (HUVECs) or **monocytes**.

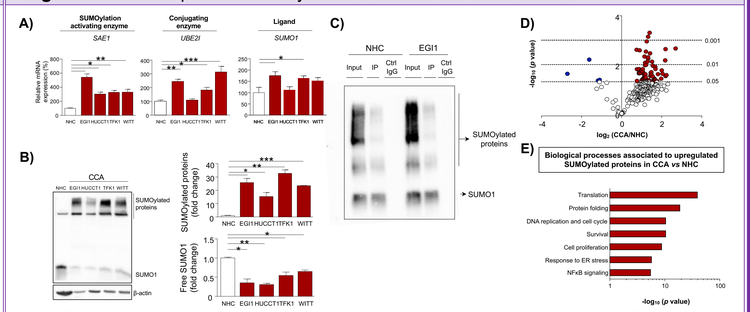
RESULTS

Figure 1: Expression levels of the SUMOylation pathway in human CCA tissue



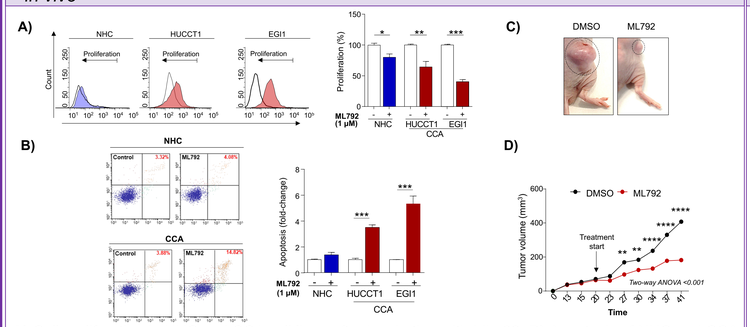
A) *SAE1 and UBE2I* mRNA expression in CCA tumors compared to NBDs and/or surrounding human liver tissue from the Copenhagen, TCGA, TIGER and San Sebastian cohorts of patients. **B)** Tumor recurrence of CCA patients (Copenhagen cohort) with low and high *SAE1* expression levels. **C)** *SAE1* mRNA expression in CCA tumors (Copenhagen cohort) grouped by tumor differentiation grade. **D)** Representative IHC images of SUMO1 and SUMO1-conjugated proteins in human liver tissues. Scale bars: 50μm.

Figure 2: Aberrant protein SUMOylation in human CCA cells *in vitro*



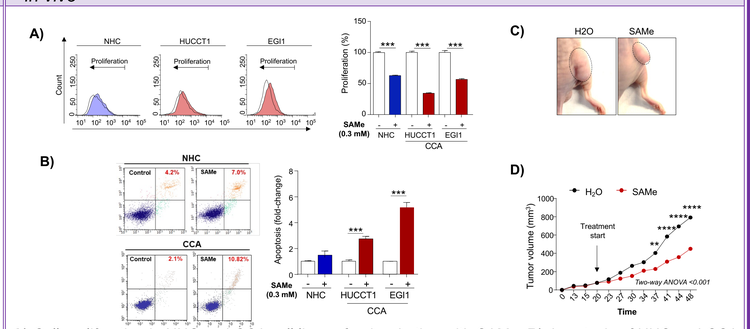
A) *SAE1*, *UBE2I* and *SUMO1* mRNA expression in NHCs and CCA cell lines. **B)** Representative immunoblot and quantification of SUMO1 and SUMO1-conjugated proteins in NHCs and CCA cells. **C)** Representative immunoblot of SUMO1-IP in NHC and CCA (EGI1) cells. **D)** Volcano plot of all identified SUMO1-IP proteins (n=310) by MS comparing fold enrichment in CCA to NHC. **E)** Proteomic analyses of significant identified proteins (n=79) between CCA and NHC by GO.

Figure 3: ML792 reduces proliferation and survival of CCA cells and halts CCA growth *in vivo*



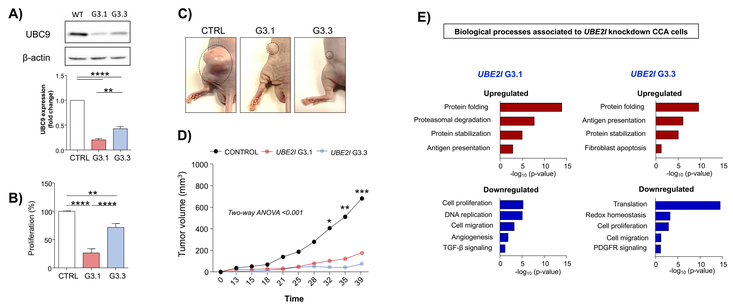
A) Cell proliferation in NHC and CCA cell lines after incubation with ML792. **B)** Apoptosis of NHC and CCA cell lines after ML792 incubation. **C)** Representative images of vehicle- or ML792-administered CCA tumors, and **D)** tumor volume growth.

Figure 4: SAMe reduces proliferation and survival of CCA cells and halts CCA growth *in vivo*



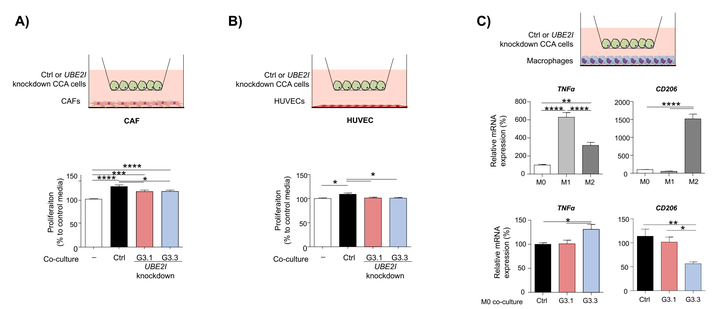
A) Cell proliferation in NHC and CCA cell lines after incubation with SAMe. **B)** Apoptosis of NHC and CCA cell lines after SAMe incubation. **C)** Representative images of vehicle- or SAMe-administered CCA tumors, and **D)** tumor volume growth.

Figure 5: CRISPR/Cas9-*UBE2I* CCA cells recapitulate SUMOylation inhibition-mediated effects on CCA



A) Representative immunoblot and quantification of WT and *UBE2I* knockdown CCA cells (clones 1 and 3). **B)** Proliferation. **C)** Representative images of Ctrl and *UBE2I* knockdown CCA tumors, and **D)** tumor volume growth. **E)** Proteomic analyses of significant differentially identified proteins in the Ctrl and CRISPR/Cas9-*UBE2I* CCA cells by GO.

Figure 6: SUMOylation impairment impacts on the crosstalk between CCA cells and their microenvironment



A) Schematic representation of co-culture experiments and proliferation of CAFs and **B)** HUVECs after co-culture with Ctrl or *UBE2I* knockdown CCA cells. **C)** *TNFα* and *CD206* (M1 and M2 macrophage markers, respectively) mRNA expression in monocytes after differentiation towards M1 or M2 macrophages and after coculture with Ctrl or *UBE2I* knockdown CCA cells.

CONCLUSIONS

Aberrant protein SUMOylation contributes to cholangiocarcinogenesis by promoting cell survival and proliferation.

Targeting protein SUMOylation reduces cell proliferation and tumor growth in experimental models of CCA.

Abnormal protein SUMOylation impacts on the CCA-stroma crosstalk.

Inhibition of SUMOylation with SAMe or **ML792** may represent a potential therapeutic strategy for patients with CCA.

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