The protease-inhibitor SERPINB3 as a critical modulator of the stem-like subset in human cholangiocarcinoma

Aim 1: SERPINB3 Expression in CCA-SPH

Expression of Intra-cellular SERPINB3

Paracrine Effect of SERPINB3

In vitro Treatment of MON Cells with Recombinant SERPINB3

Recombinant SERPINB3, 6 days

Intracellular Effect of SERPINB3

Stable Transfection of MON Cells with SERPINB3

Empty vector

SERPINB3 vector

Aim 2: In Vitro Paracrine and Intracellular Effect of SERPINB3

Experimental Design

Gene Expression Profile

MON\textsuperscript{SERPINB3} showed significant overexpression of genes associated with stemness, pluripotency, EMT, and ECM remodeling. Of note, the same genes were not affected by exposure of MON to exogenous rSERPINB3.

Aim 3: In Vivo Tumorigenic Role of SERPINB3

SB3 tumorigenic capacity in NSG mice

MON\textsuperscript{SERPINB3} were highly tumorigenic and engrafted earlier compared to MON\textsuperscript{CTR}.

MON\textsuperscript{SERPINB3}-derived tumors had higher weight compared to MON\textsuperscript{CTR}-derived tumors.

Expression of SB3 mRNA was higher in tumors than in matched surrounding liver

SB3 mRNA expression

Analysis of OS and TTR in High- and Low-SB3 Patients

Patients with high SB3 expression had a significantly lower survival (Log-rank curve) and shorter time to recurrence (Gehan-Breslow-Wilcoxon curve) than those with low SB3

Aim 4: Analysis of SB3 Expression in Human CCA Samples and Correlation with Clinical Outcomes

SB3 Protein Expression in Paraffin-Embedded Human CCA Samples

Variable degrees of SB3 expression were observed. Patients with SB3 high scores had a three-fold lower time to recurrence compared to those with low SB3 expression

Conclusions

In conclusion, our data provide evidence for SB3 as a new molecule potentially involved in the biology of the CCA stem-like compartment, possibly supporting tumor fate and CSC formation. Based on these results, interference with SB3-generated signals should be further investigated for its potential therapeutic relevance, and SB3 expression in CCA deserves to be analyzed as a prognostic biomarker in larger series of patients.