# CRENIGACESTAT INHIBITS iCCA TUMOR PROGRESSION VIA NOTCH1/HES1/CD90 PATHWAY


**Serena Mancarella[1], Isabella Gigante[1], Grazia Serino[1], Diego Calvisi[2], Antonio Cigliano[2], Silvia Ribback[3], Rodolfo Sardone[1], Raffaele Armentano[1], Gianluigi Giannelli[1]**

1National Institute of Gastroenterology "S. de Bellis", Research Hospital, Castellana Grotte, Italy
2Institute of Pathology, University of Regensburg, Regensburg 93053, Germany
3Institute of Pathology, University of Greifswald, Greifswald 17489, Germany

I.R.C.C.S. "Saverio de Bellis"
Ente Ospedaliero
Specializzato in Gastroenterologia
Castellana Grotte

*Serena Mancarella, PhD*
*IRCCS «s. De Bellis» Personalized Medicine Laboratory*
*Via TURI, 27 Castellana Grotte(BA),Italy*
*Email: serena.mancarella@irccsdebellis.it*


## ABSTRACT


Intrahepatic Cholangiocarcinoma (iCCA) is a malignant tumor arising from intrahepatic bile ducts[1] often diagnosed belatedly. Currently, curative treatment involves partial hepatectomy. In patients with early stage disease, chemotherapy and radiation treatments have been accepted as standard first-line treatment, with some partial benefit. Detection of novel diagnostic and prognostic biomarkers may make as early prognosis and potential treatment in iCCA. Previously, we have demonstrated the involvement of Notch1 receptor in iCCA progression by developing and validating an iCCA patient-derived xenografts (PDX) model[2]. Specifically, we have demonstrated the effect of Crenigacestat, a gamma-secretase inhibitor (GSI), that significantly inhibited Notch pathway and tumor growth. By gene expression analysis, we have proved that several genes implied in Notch-dependent angiogenesis, were down-regulated by Crenigacestat treatment[2]. Another gene that, in iCCA PDX model, undergoes the same effect following GSI treatment is THY1/CD90. In the current study, we evaluated THY1/CD90 gene and protein expression in human iCCA tissues and PDX model. THY1/CD90 co-localizes with epithelial marker EPCAM, showing no doubts on its origin. Furthermore, by Notch1 silencing in human iCCA cell lines, we found that THY1 gene was down-regulated, as well as HES1, indicating its expression was related to their one. We further demonstrate that RBPJ, an important transcriptional regulator in Notch signaling pathway, has largely overlapping binding sites on THY1 promotor, assuming that it acts as a downstream target for Notch signaling. Finally, Kaplan-Meier survival analysis of human iCCA tumor patients demonstrated that NOTCH/HES1/THY1 expressing tumors are correlated to a worse iCCA patient survival. We herein summarize that NOTCH/HES1/THY1 axis can be uses to guide the diagnosis and treatment of iCCA patients.


**REFERENCES**


1. Razumilava N et al. Cholangiocarinoma. Lancet 2014 Jun 21;383(9935): 2168-79
2. Mancarella S, et al. Crenigacestat, a selective NOTCH1 inhibitor, reduces intrahepatic cholangiocarcinoma progression by blocking VEGFA/DLL4/MMP13 axis. Cell Death Differ. 2020 Aug;27(8):2330-2343.


## AIM

To identify multiple pathway potentially actionable for personalized therapy in iCCA patients.

## RESULTS

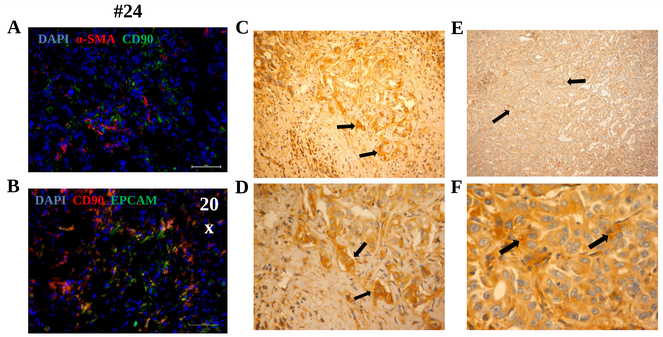


**Figure 1. CD90 staining in PDX model and human tissues**. (A). CD90 does not co-localize with α-SMA (red), (B) but it does with EpCam (green) on frozen tissues of PDX as showed by immunofluorescence. CD90 immunohistochemistry on human iCCA paraffin embedded tissues at low (x4) and high (x20) magnification. An intense cytoplasmic positivity in approximately 60% of the cells independently by the moderate (C-D) or severe (E-F) differentiation.

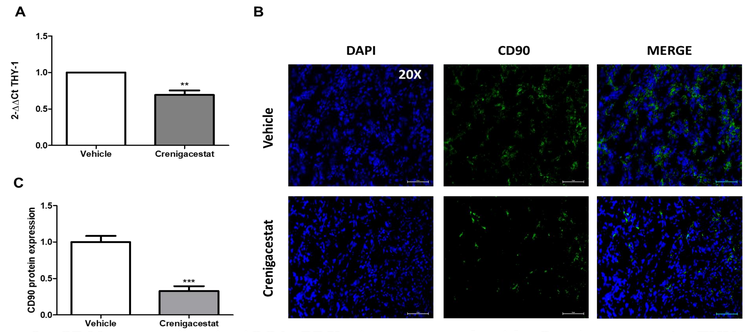


**Figure 2. CD90 expression in iCCA PDX tissues treated with Crenigacestat.** A. THY1 is downregulated following treatment with Crenigacestat in iCCA PDX mice. Fold-change -2.49 and FDR= 0.01. B. Representative images with immunofluorescence staining show downregulation of CD90 expression (low panel). C. Staining quantification was calculated as mean of three images per tissue of PDX mice treated with Crenigacestat compared to vehicle. Mean ± SEM (number of PDX mice treated with vehicle = 10, number of PDX mice treated with Crenigacestat = 10 ). ***p < 0.001 calculated with Mann Whitney test. Magnifications: ×20.

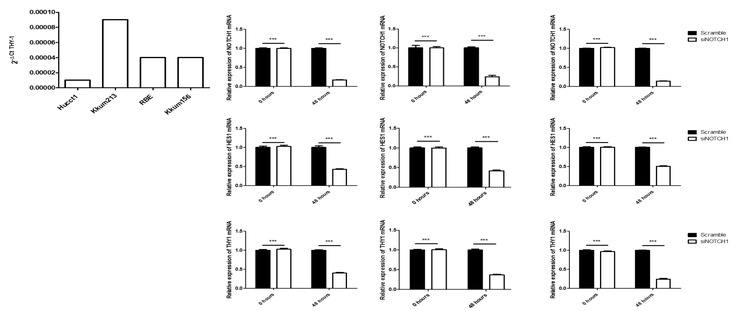


**Figure 3. THY1 expression in human iCCA cell lines and its correlation with Notch pathway**. A. Relative expression of THY1 mRNA shows high THY1 expression in KKUM213, RBE and KKUM156 cell lines and weak THY1 expression in HUCCT1 cell line. B. Notch1 silencing in KKUM-156, KKUM-213 and RBE human iCCA cell lines leads to a significant decrease of THY1 mRNA expression.

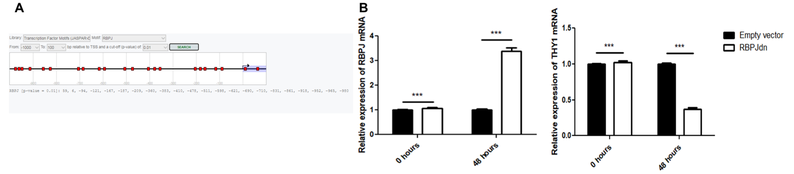


**Figure 4. RBPJ specifically binds THY1 promotor gene.** A. In-silico prediction of RPBJ binding sites on THY1 gene promoter through the EPDnew software, using a cut-off of P-value of 0.01. Binding sites are represented in red squares. B. Notch pathway was inhibited by the transient overexpression of a dominant negative form of the NOTCH transcriptional co-activator RBPJ (dnRBPJ). After transfection, THY1 gene expression level was significantly reduced (***p < 0.001 ) showing that THY1 expression was downstream to NOTCH pathway

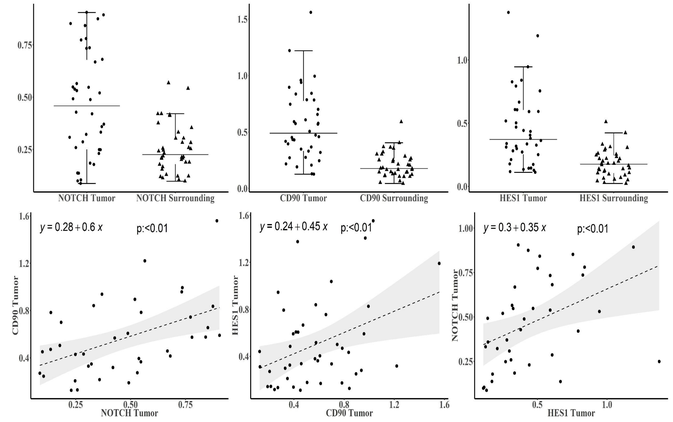


**Figure 5. Jitter Boxplots and scatterplots of NOTCH, CD90 and HES1.** A. Boxplots of different quantity of biomarkers (y axis) by tumor and tumor sourrounding localization (x axis), showing jitter distribution (dot for tumor and triangle for sourrounding), median and interquartile range differences. B. Scatterplots of correlation between different tumor markers (NOTCH, CD90 and HES1) showing trend line (dotted line) and confidence interval around that (gray area) and scatter interpolated distribution of the quantities (dots).

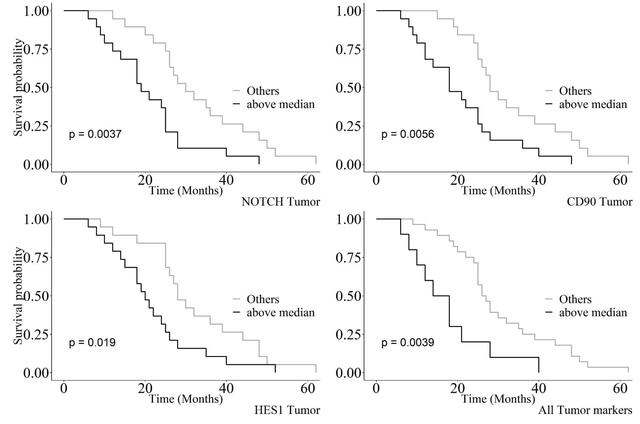


**Figure 6. Kaplan Meier survival plots.** Kaplan Meier survival plots to show survival proportion of subjects above median for each biomarkers (grey lines) vs subjects below median (black line) on y axis and survival time (months) on the x axis.

## CONCLUSION

In conclusion, these results suggest that the NOTCH1/HES1/CD90 axis could play a significant role in enhancing the effectiveness of GSI in the treatment of iCCA patients.