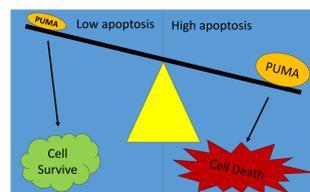




## INTRODUCTION

FAS is the key component of the extrinsic death pathway that can induce apoptosis when binds to its cognate ligand FASL.

p53-upregulated modulator of apoptosis (PUMA) is a potent pro-apoptotic protein involves in p53-dependent and independent apoptosis pathway. However, whether PUMA play a role in the regulation of FAS in cancer is still unclear. This study reports on a relevant function of FAS and PUMA in hepatocellular carcinoma (HCC) cellular death.

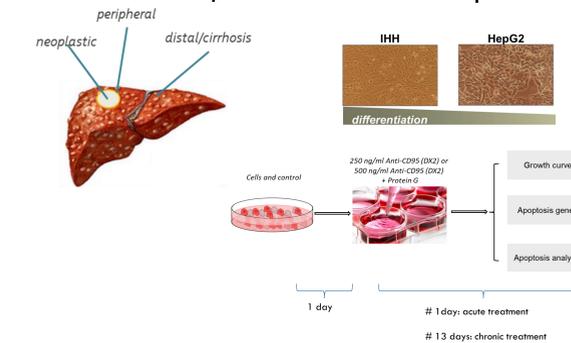


## METHODS

*In vivo*: the mRNA analysis of FAS, FASL, and PUMA were collected from 111 patients undergoing liver resection without any prior treatments (39 HCC, 30 peri-HCC, 31 distal/cirrhosis); liver donors were used as control (11 normal).

*In vitro*: human immortalized hepatocyte cell line IHH and HCC cell line HepG2 were used. Apoptosis-induction was performed by using anti-FAS (DX2) at concentrations of 250 ng/ml for 1 until 13 days. Flow cytometry was performed for FAS/FASL positivity and Annexin-V- PI apoptosis test, quantitative real-time PCR for mRNA expressions, and growth curve test for cells viability.

*In vivo*: human tissues specimens *In vitro*: human hepatic cell lines



## RESULTS

### *In vivo* study

FAS AND FASL were expressed in hepatic tissues and were upregulated in HCC compared to cirrhosis and normal (\*p<0.05, \*\*p<0.01 vs normal)

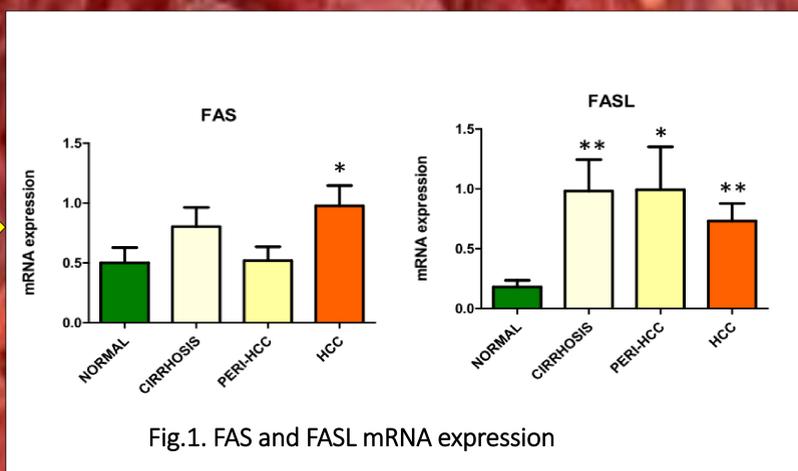


Fig.1. FAS and FASL mRNA expression

### *In vitro* study

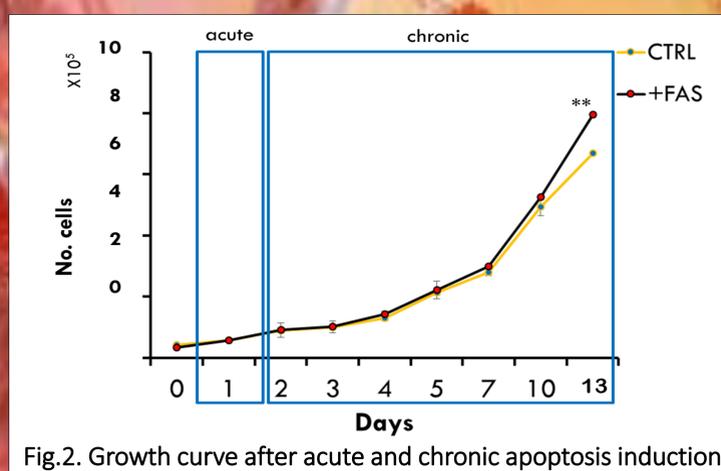


Fig.2. Growth curve after acute and chronic apoptosis induction

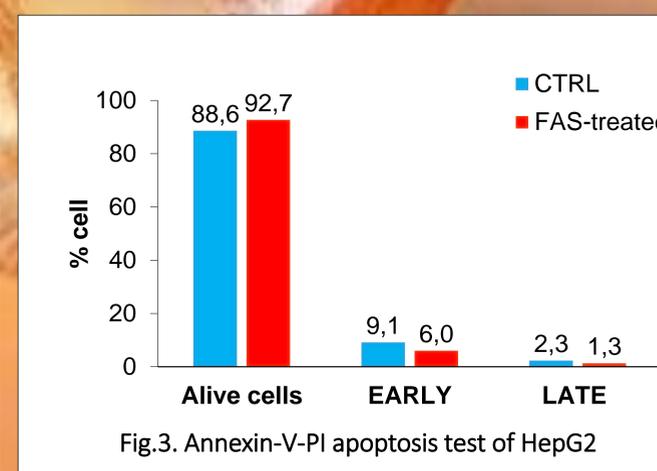


Fig.3. Annexin-V-PI apoptosis test of HepG2

HepG2 cells treated with FAS antibody become more resistant to apoptosis

PUMA deficiency in cancer samples suggests its pro-apoptotic functions

Samples	Positive (n)	Positive (%)
Cirrhosis	24/31	77
Peri-HCC	19/27	70
HCC	13/32	41

Table 1. PUMA expression in tissue samples

The growth of HepG2 cells was increased after chronic induction by FAS antibody (\*\*p<0.001). FAS can induced the cells/tumor growth

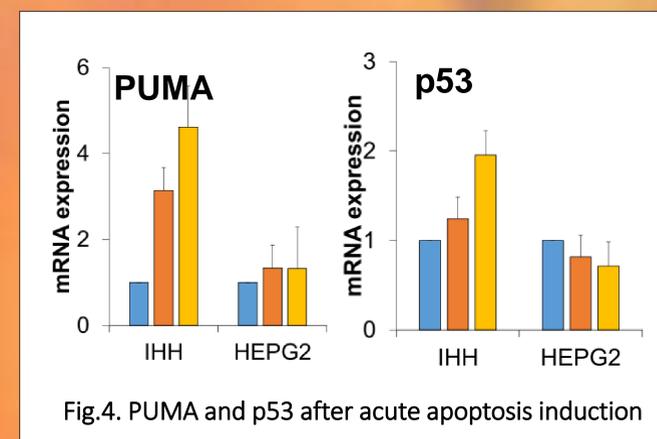


Fig.4. PUMA and p53 after acute apoptosis induction

PUMA mRNA was up regulated only in hepatocyte cell line  
 No PUMA No Cell-Death

## CONCLUSION

HCC might have resistant mechanisms against FAS-dependent apoptosis. Deficiency of PUMA in HCC indicates its important function in apoptosis pathway. Deregulation of FAS and PUMA are involved in HCC development.

## REFERENCES

- Peter, M.E and Kramer, P.H. The CD95 (APO-1/Fas) DISC and beyond. Cell Death and Differentiation. 2003: 26–35
- Yu, Jian and Zhang, Lin. No PUMA, no death: Implications for p53-dependent apoptosis. Cancer Cell. 2003: 248-249