Increased hepatic pregnane X receptor protein expression negatively correlates with tight junction proteins in patients with hepatocellular carcinoma

Balasubramaniyan V[1], Tanya Mishra[1], Amit Kumar Ram[1] and Biju Pottakkat[2]

Department of Biochemistry, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Pondicherry-605006, India.

Background

- The nuclear pregnane X receptor (PXR) is a master regulator of detoxification and plays a key role in xenobiotic metabolism
- CYP3A4 - most abundant hepatic and intestinal phase I enzyme, metabolizing 50% of clinically used drugs in humans
- In hepatic cancer PXR function is still poorly defined.
- Tight junctions (TJ) are intercellular adhesion complexes - essential to the barrier function of epithelia and endothelia.
- TJ proteins regulate several key signaling pathways in cancer.
- Decreased TJ proteins expression promotes tumor growth and metastasis in ovarian and breast cancers.
- On the other hand, high expression of TJ proteins suppresses invasion and metastasis in pancreatic cancer.
- Furthermore, deranged tight junction proteins in HCC leads to tumour dissemination and progression of HCC.

Aim

This study aimed to identify an association between inflammation and PXR levels in HCC. We also investigated the expression of PXR and tight junction proteins in HCC.

Methods

Following Institutional ethical committee approval, a total of 80 individuals (40 HCC cases and 40 normal healthy controls) were enrolled in the study. Baseline characteristics, biochemical parameters and alpha-fetoprotein (AFP) were analysed by Beckman Coulter autoanalyzer. Estimation of serum PXR and IL-1B were done by ELISA. Hepatic PXR and tight junction proteins expressions were analysed by western blotting and immunohistochemistry.

Statistical analysis

Data were analysed by t test-Mann Whitney U test; $p<0.05$ was considered statistically significant. Results are presented as mean ± SEM using GraphPad Prism 7.0.

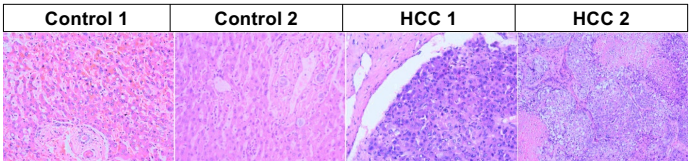
Results

Table 1 Comparison of biochemical parameters in healthy controls and HCC patients

Parameters	Healthy controls(n=40) Mean ± SEM	HCC patients (n=40) Mean ± SEM
Total bilirubin (mg/dL)	0.47 ± 0.048	5.57 ± 1.11***
Direct bilirubin (mg/dL)	0.18 ± 0.015	2.69 ± 0.57***
Total protein (g/dL)	6.638 ± 0.156	6.81 ± 0.16
Albumin (g/dL)	3.625 ± 0.113***	3.048 ± 0.11
AST(IU/L)	27.05 ± 1.639	188.6 ± 26.67***
ALT (IU/L)	31.03± 3.414	85.66 ± 18.87***
ALP (IU/L)	80.95 ± 6.636	525.2± 134.4***
GGT (IU/L)	36.63 ± 5.774	409.2± 114.7***
PT (sec)	11.69 ± 0.81	15.87 ± 1.05***
INR	1.02 ± 0.024	1.305 ± 0.09***
Urea (mg/dL)	19.05 ± 1.476	24.23 ± 1.828
Creatinine (mg/dL)	0.73 ± 0.046	0.77 ± 0.05
Sodium (mEq/L)	131.9 ± 1.408	132.3 ± 0.768
Potassium (mEq/L)	3.54 ± 0.08	3.67 ± 0.07
AFP (ng/ml) Median (IQR)	12.0 (10.0 – 15.75)	194.0 (30.0 – 1493.0)***

Values are expressed as mean ± SEM. Mann–Whitney test was performed and p <0.05 was considered as statistical significant. Note: ***p < 0.0001 compared to control subjects. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transferase; ALP, alkaline phosphatase; PT, prothrombin time; INR, International normalized ratio; AFP, alpha fetoprotein.

Fig 1. Hepatic pathological changes observed by H&E (20X) in control and HCC patients.



Control livers show Hepatocytes with densely stained cytoplasm with prominent granularity, round nuclei and nucleoli, arranged in regular cords or plates separated by sinusoids. Portal zone includes connective tissue, hepatic artery, portal vein and bile duct. HCC livers show malignant cells, well differentiated and interdigitated with scant basophilic cytoplasm, nuclear overcrowding and hyperchromatic nuclei. Cords in HCC liver tissue are wide apart compared to that of control liver.

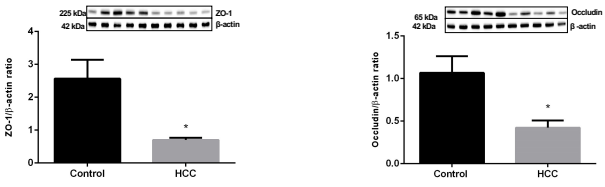


Fig 2. Hepatic ZO-1 and Occludin proteins expression by Western blotting.

When compared to control liver, hepatic ZO-1 and occludin proteins expression are decreased in HCC liver. Values are expressed as mean ± SEM. Mann–Whitney test was performed and p <0.05 was considered as statistical significant. Note: *P<0.05 versus control liver.

Conclusions

- ❖ Our novel findings indicate that increased inflammation is associated with the upregulation of PXR and downregulation of tight junction proteins in HCC.
- ❖ Increased PXR in HCC could be a protective factor but further mechanistic studies are warranted.
- ❖ Targeting PXR and tight junction proteins could be a useful approach to facilitate HCC treatment.

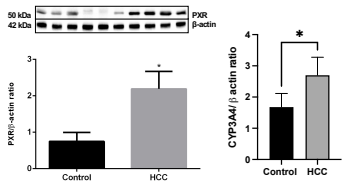


Fig 3. Hepatic PXR and CYP3A4 proteins expression by Western blotting.

When compared to control liver, hepatic PXR and its target enzyme CYP3A4 proteins expression are increased in HCC liver. Values are expressed as mean ± SEM. Mann–Whitney test was performed and p <0.05 was considered as statistical significant. Note: *p < 0.05compared to control subjects.

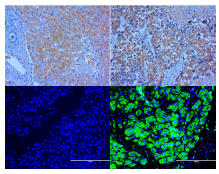


Fig 4. Cellular PXR expression in control and HCC.

Control liver shows moderate cytoplasmic and focal nuclear positive. HCC liver shows strong cytoplasmic and focal nuclear positive. (200x)

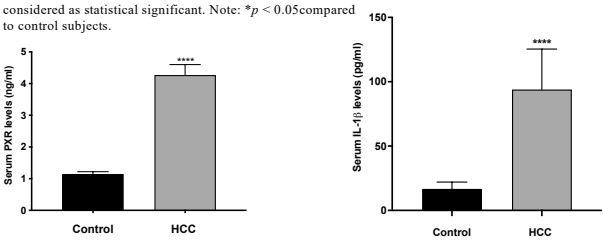


Fig 5. Serum PXR and IL-1β levels in control and HCC.

When compared to control, serum PXR and IL1 beta concentrations are increased in HCC patients. Values are expressed as mean ± SEM. Mann–Whitney test was performed and p <0.05 was considered as statistical significant. Note: ****p < 0.0001 compared to control subjects.

Fig 6. ROC curve: PXR

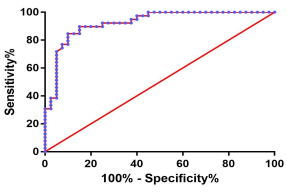


Fig 7. Correlation between PXR and IL1-β

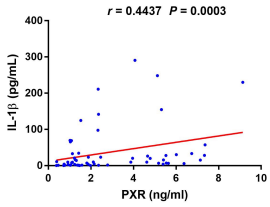


Table 2. ROC curve of PXR

Parameter	Cut-off	AUC	Sensitivity	Specificity	Likelihood Ratio
PXR	>2.014 (ng/mL)	0.9247	84.62%	90%	8.462

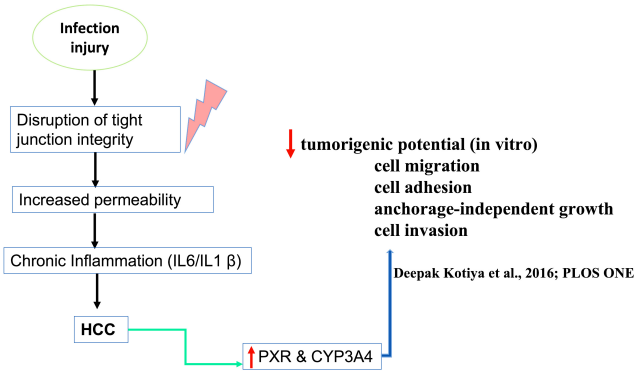


Fig.8. Proposed role of inflammation in regulating tight junction integrity and PXR in HCC.