Gain of positive charges in HBsAg C-terminus correlates with HBV-induced liver cancer

Romina Salpini[1], Luca Carioti[1], Marianna Aragri[1], Domenico Di Carlo[1], Luna Colagrossi[1], Giuseppina Cappiello[2], Pascale Trimoulet[3], Herve Fleury[3], Simona Francioso[4], Loredana Sarmati[5], Massimo Andreoni[5], Mario Angelico[4], Francesca Ceccherini-Silberstein[1], Carlo-Federico Perno[1], Valentina Svicher[1]

[1] Tor Vergata University, Department of Experimental Medicine and Surgery, Rome, Italy, [2] Microbiology and Virology Unit, "S. Pertini" Hospital, Rome, Italy, [3] Hôpital Pellegrin tripode, Laboratoire de Virologie, Bordeaux, France, [4] Tor Vergata University Hospital, Hepatology Unit, Rome, Italy [5] Tor Vergata University Hospital, Infectious Diseases Unit, Rome, Italy

BACKGROUND

- Acquisition of positively charged amino acids (aa) can affect the folding of a transmembrane protein domain.
- HBsAg C-terminus is a hydrophobic transmembrane domain, composed by alpha-helices, critical for proper HBsAg secretion. Altered HBsAg folding in endoplasmic reticulum (ER) membrane can affect HBsAg secretion and in turn promote HBV-induced hepatocellular carcinoma (HCC).
- The role of mutations associated with gain of charged aa in HBsAg C-terminus on HBV-induced HCC onset is not known

OBJECTIVES

The main goals of this study are:

- To define the correlation between the acquisition of a positively charged amino acid in HBsAg C-terminus and HCC onset in chronically HBV infected patients.

-To characterize the impact of these positively charged amino acids on the putative three-dimensional HBsAg C-terminus structure

MATERIALS & METHODS

- This study includes 807 HBV chronically infected patients from routine clinical practice: 28 with HCC (78.6% D; 21.4% A), and 779 patients without HCC (79.8% D; 20.2% A).
- Mutations associated with gain of charged aa in HBsAg C-terminus (aa189-226) are analyzed. Association of mutations with HCC is assessed by multivariable logistic regression model.
- Hydrophobicity profiles of HBsAg C-terminus are constructed to predict stability of a domain in a membrane (Black, 1991).
- I-Tasser is used to predict three-dimensional HBsAg structures (aa:1-226) and their stability (ΔΔG[wt-mutated]<0 indicating reduced stability in presence of mutation based on Quan,2016).

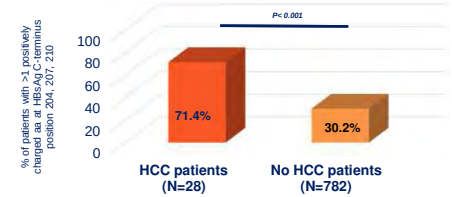
RESULTS

Patients' characteristics	Patients with HCC (N=28)	Patients without HCC (N=779)
Male, N(%)	25 (89.3)	541 (69.4)
Italian Nationality, N(%)	21 (75)	595 (76.4)
Median Age [Years] (IQR)	64 (57-70)	48 (37-59)
HBeAg negative, N(%)[a]	17 (77.3)	
Median HBV-DNA [log IU/ml] (IQR)	4.0 (2.4-5.7)	3.8 (2.7-5.4)
Median ALT [IU/L] (IQR)	67 (29-106)	42 (28-80)
Median AST [IU/L] (IQR)	50 (34-109)	31 (22-59)
HBV genotype		
D gen, N(%)	22 (78.6)	622 (79.8)
A gen, N(%)	6 (21.4)	157 (20.2)
Anti-HBV antiviral treatment		
Naive, N(%)	11 (39.3)	464 (59.5)
Treated, N(%)	17 (60.7)	315 (40.4)

a The percentages were calculated on 22 HCC and control patients with an available HBeAg status.

RESULTS

The gain of ≥1 positively charged aa at HBsAg C-terminus positions 204, 207, and 210 tightly correlates with HCC



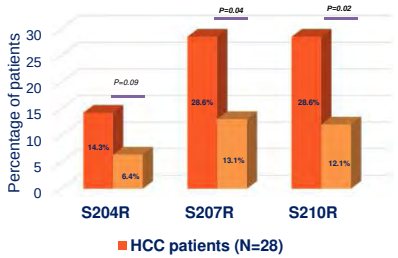
The histogram reports the prevalence of patients with ≥1 positively charged aa at HBsAg C-terminus positions 204, 207, and 210 in pts with and without HCC. Statistically significance was determined by Fisher Exact test.

Variables	Univariate analysis		Multivariate analysis	
	crude OR [95% CI]	p-value	adjusted OR [95% CI]	P value
Gender (female vs. male[a])	**3.62 (1.08-12.11)**	**0.037**	2.97 (0.85-10.39)	0.089
Age (per 1 year increase)	**1.09 (1.06-1.13)**	**<0.0001**	**1.10 (1.06-1.14)**	**<0.0001**
Naïve vs experienced[a]	1.10 (0.52-2.37)	0.798	1.05 (0.46-2.44)	0.902
HBV-DNA (per 1 log_{10} IU/mL)	1.03 (0.83-1.27)	0.802	0.99 (0.79-1.24)	0.934
ALT	0.99 (0.98-1.01)	0.609	0.99 (0.98-1.01)	0.098
AST	1.01 (0.99-1.03)	0.226	1.01 (0.99-1.03)	0.069
Genotype (D vs A[a])	0.93 (0.37-2.32)	0.869	0.53 (0.18-1.57)	0.249
At least one mutation (S204R, S207R and S210R)	**5.79 (2.51-13.33)**	**<0.0001**	**6.32 (2.60-15.35)**	**<0.0001**

[a] Reference group

Multivariable analysis confirms this correlation correcting for patients' demographics, HBV genotype, serum HBV-DNA and anti-HBV drugs use (OR[95%CI]:6.3[2.6-15.3], P<0.001).

The gain of positively charged aa derives from mutations S204R, S207R and S210R present in 14.3%, 28.6% and 28.6% of HCC-patients



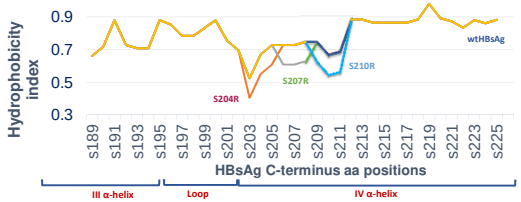
The histogram shows the prevalence of mutations associated with the gain of a positive charge in HBsAg C-terminus, calculated in HCC (N=28) and no HCC patients (N=728). Statistically significant differences were assessed by Fischer Exact Test.

S204R, S207R and S210R determine a reduction in HBsAg C-terminus stability



The histogram shows the variation of HBsAg C-terminus stability in presence of each mutation associated with HCC. HBsAg C-terminus stability was measured by calculating ΔΔG (mutated - WT) [STRUM (Quan et al., Bioinformatics, 2016)]

S204R, S207R and S210R determine a reduction in hydrophobicity index of HBsAg C-terminus compared to wt



Moreover, S204R, S207R and S210R determine a shortening of membrane-spanning alpha-helix motif compared to wt

HBsAg MUT	alpha helix 3 (START-STOP)	LOOP (START-STOP)	alpha helix 4 (START-STOP)	BENT (START-STOP)
WT	P188 - M198	W199 - S204	L205 - Y225	L213-P214
S204R	P188 - W199	Y200 - I208	L209 - Y225	L213-P214
S207R	P188 - M198	W199 - I208	L209 - Y225	L213-P214
S210R	P188 - M198	W199 - I208	L209 - Y225	L213-P214

CONCLUSIONS

Gain of positively charged aa at specific HBsAg C-terminus positions tightly correlates with HCC, by altering the proper folding of this domain in ER membrane. These mutations might affect HBsAg secretion and in turn contribute to the initiation of HBV-related tumorigenesis. Their role in identifying patients at higher HCC-risk deserves further investigation

ACKNOWLEDGMENTS

HIRMA PROJECT

Bandiera, PB05 PROJECT

AVIRALIA Foundation