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Supplementary Materials for

Androgen Receptor Promotes Hepatitis B Virus–Induced Hepatocarcinogenesis Through Modulation of Hepatitis B Virus RNA Transcription

Ming-Heng Wu, Wen-Lung Ma, Cheng-Lung Hsu, Yuh-Ling Chen, Jing-Hsiung James Ou, Charlotte Kathryn Ryan, Yao-Ching Hung, Shuyuan Yeh, Chawnshang Chang*

*To whom correspondence should be addressed. E-mail: chang@urmc.rochester.edu

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Materials and methods

Serum testosterone concentration and tissue preservation

We sacrificed mice at the indicated time points, drew 1 ml of blood by cardiocentesis and immediately assayed for serum testosterone level using the Coat-A-Count Total Testosterone radioimmunoassay (Diagnostic Products).

Microarray Sample Preparation and Labeling

The samples for microarray analysis were done according to standard protocol (Nimblegen). In brief, double-stranded cDNA from 10μg of total RNA was performed using Invitrogen's SuperScript™ Double-Stranded cDNA Synthesis Kit, and then the cDNA was treated with RNase A, the total RNA was cleared by phenol/chloroform/isoamyl alcohol and precipitated with ammonium acetate/glycogen/ethanol. Gel analysis was used to verify the Double-Stranded cDNA step that showed cDNA sample had a smear band of 500–2000bp. The reactions are then labeled with Cy3-9mer Primers by Klenow enzyme, followed with precipitation using NaCl and isopropanol. The precipitation was re-suspended in 25 μl deionized water.

Microarray hybridization and data analysis

Microarrays hybridization was combined with 4 μg each of the sample and NimbleGen Hybridization Kit (NimbleGen Systems) was added for the hybridization reaction. Hybridization reaction was performed in MAUI Hybridization System (BioMicro). After the hybridization, the array was washed and dried according to the NimbleGen Washing Kit (NimbleGen Systems) protocol. The array image was acquired with an Axon 4000B laser

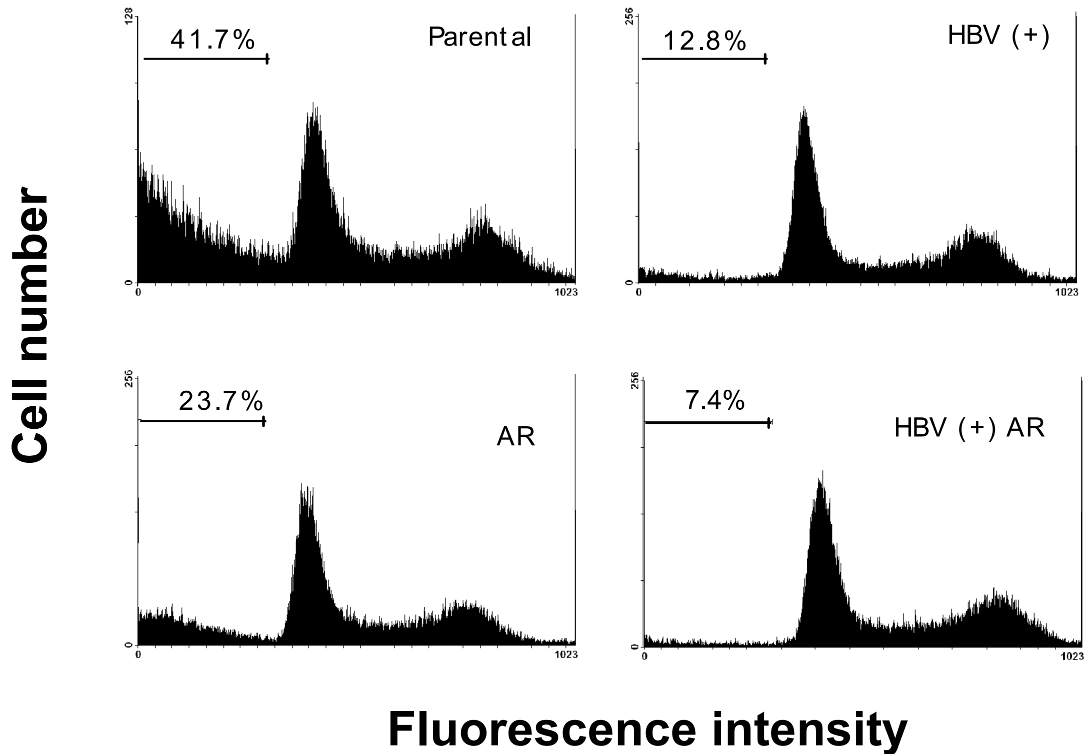
scanner at 5 μm resolution and intensity data was extracted using the software NimbleScan (NimbleGen Systems). The data was further examined using NexuExp software (BioDiscovery). Gene expression changes with more/less than one fold compared with the control group and with $p<0.05$ were considered statistically significant differentially expressed genes between samples.

PCR array analysis

Total RNA were extracted from mice liver tumors and converted to cDNA by RT^2 First Strand kit (SABiosciences, MD, USA). Mouse oncogenes and tumor suppressor genes expression were examined by PCR array according to the manufacturer's instructions (SABiosciences, MD, USA)(Cat.PAMM-502).

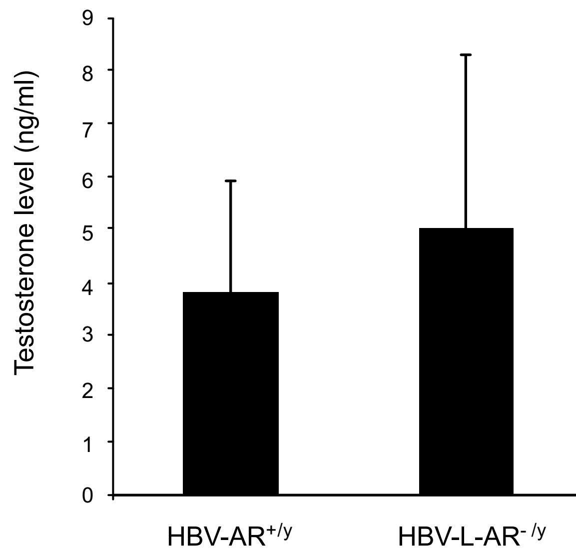
Quantitative Real Time PCR (qRT-PCR)

Total RNA was extracted using TRIzol reagent according to the manufacturer's instructions (Invitrogen). 4 μg total RNA was reverse transcripted to cDNA by IMProm-II Reverse Transcriptase (Promega). Expression of different mRNAs was analyzed by qRT-PCR as previously described (1).

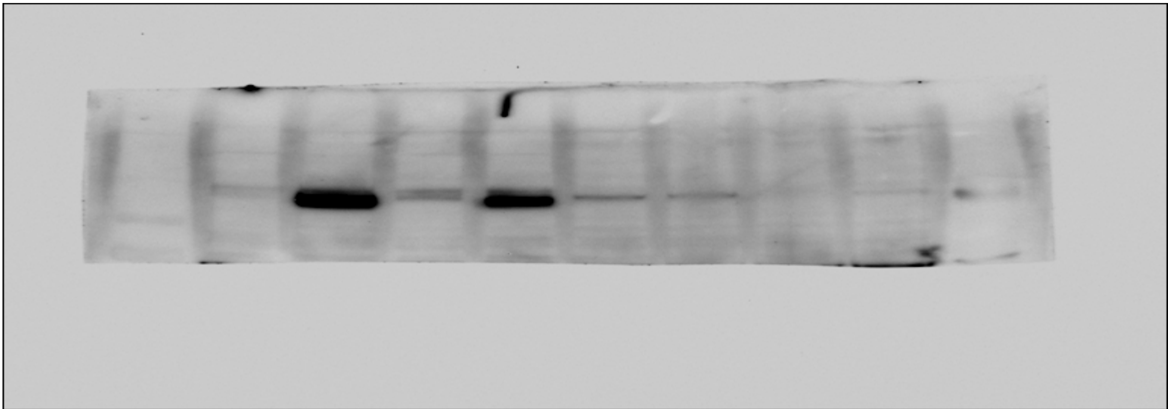


Suppl. Fig. S1. AR and HBV cooperatively reduce stress-induced apoptosis in HepG2 cells. Cells were subjected to one exposure of UV (50 J/m^2), harvested 24 hr, and then fixed in 70% ethanol. The cells were stained with propidium iodide (40 mg/ml in PBS). Apoptotic cells were quantified by FACScan with CELLQuest software (Becton Dickinson) and presented as the percentage of hypodiploid cells.

Suppl. Fig. 2, Wu. et al.



Suppl. Fig. S2. Loss of hepatic AR does not influence serum testosterone levels in the HBV-$AR^{+/y}$ and HBV-L-$AR^{-/y}$ mice. The sera from 22-week-old HBV-$AR^{+/y}$ and HBV-L-$AR^{-/y}$ mice with low dose DEN injection were collected and measured testosterone levels by ELISA assay. There is no significant difference of serum androgen levels between two groups of mice.



Suppl. Fig. S3. Full Western blot of AR expression in HepG2 cells.

Suppl. Table 1, Wu, et al.

Assays	gene name	fold changed
cDNA microarray	Fos	9.02
	Met	3.86
	Smad1	3.11
	ccnd1	3.1
	Stat3	3.08
	Ets1	3.03
	Caspase 8	2.92
	Smad4	2.53
	Mcl1	2.72
	Myc	2.39
Oncogene PCR array	Fos	29.47
	Runx1	4.41
	Prkca	2.87
	Tnf	2.5
	Mycn	2.49
	Junb	2.23
	Myc	2.17
	Kitl	2.12
	ccnd1	1.71
	cdh1	1.68
Real-time PCR	Fos	9.93
	Igf2	3.12
	Myc	2.94
	Bcl-xl	2.22
	ccnd1	1.89

Red color indicates the gene consistently decreased in HBV-L-AR$^{-/y}$ mice livers in three assays

Suppl. Table 1. Expressions of HCC associated genes are suppressed in HBV mice with loss of hepatic AR. Oncogene expressions in HBV-L-AR$^{-/y}$ and HBV-AR$^{+/y}$ tumors were measured by cDNA microarray, oncogene PCR array (SABiosciences), and quantitative real-time PCR. The significantly down-regulated oncogenes in HBV-L-AR$^{-/y}$ tumors from the three assays are listed.

Reference

1. Z. Yang, Y. J. Chang, H. Miyamoto, S. Yeh, J. L. Yao, P. A. di Sant'Agnese, M. Y. Tsai & C. Chang. Suppression of androgen receptor transactivation and prostate cancer cell growth by heterogeneous nuclear ribonucleoprotein A1 via interaction with androgen receptor coregulator ARA54. Endocrinology 148, 1340-1349 (2007).