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RAPID COMMUNICATION

Liver expression of steroid hormones and Apolipoprotein D receptors in hepatocellular carcinoma

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Abstract

AIM: To evaluate the tissular expression of Androgen (A), Estrogen (E) and Progesterone (Pg) receptors, and Apolipoprotein D (ApoD), in liver tumors from resected hepatocellular carcinoma (HCC) cases in order to assess their possible relationship to prognosis.

METHODS: We performed an immunohistochemical study using tissue microarrays (containing more than 260 cancer specimens, from 31 HCC patients and controls) to determine the presence of specific antibodies against AR, ER, PgR and ApoD, correlating their findings with several clinico-pathological and biological variables. The staining results were categorized using a semi-quantitive score based on their intensity, and the percentage of immunostained cells was measured.

RESULTS: A total of 21 liver tumors (67.7%) were positive for AR; 16 (51.6%) for ER; 26 (83.9%) for PgR and 12 (38.7%) stained for ApoD. We have found a wide variability in the immunostaining score values for each protein, with a median (range) of 11.5 (11.5-229.5) for AR; 11.1 (8.5-65) for ER; 14.2 (4-61) for PgR; and 37.7 (13.8-81.1) for ApoD. A history of heavy ethanol consumption, correlated positively with AR and PgR and negatively with ER status. HCV chronic infection also correlated positively with AR and PgR status. However, the presence of ApoD immunostaining did not correlate with any of these variables. Tumors with a positive immuno-staining for PgR showed a better prognosis.

CONCLUSION: Our results indicate a moderate clinical value of the steroid receptor status in HCC, emphasizing the need to perform further studies in order to evaluate the possible role of new hormonal-based therapies.

Key words: Androgen; Estrogen; Progesterone and Apolipoprotein D receptors; Hepatocellular carcinoma; Tissue micro-arrays

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the second cause of tumor-related deaths worldwide[1]. Its incidence is high in several geographical areas mainly located in Africa and Asia and is expected to increase in developed countries at short term, mainly due to the increasing number of chronic HCV infections observed in the last decades[2]. In addition, its prognosis remains dismal, despite several treatment options being nowadays available, since the estimated 5 years survival rate is less than 50% and the disease-free survival is around 30%[3]. Moreover, systemic chemotherapy has not shown good results yet.

There are several studies suggesting that HCC might be a hormone-responsive neoplasm[4,5], and the role of sex hormone receptors in primary liver tumors have been studied experimentally[6,7]. Androgen receptors (AR) have been determined in HCC and in non-tumoral liver tissue, its expression being more intense in cancerous cells[8]. In addition, there is little information about the role of estrogen and progesterone receptors in HCC, since their presence has been demonstrated using different techniques, between 23%-48% and 14.3%-18% of tumors, respectively[9,11]. On the basis of this evidence, several clinical trials have investigated the potential role of antiestrogenic agents (tamoxifen), in increasing survival in HCC patients. However, these studies have shown conflicting and non-promising results[12].

Recently, some studies have shown a possible

protective role of Apolipoprotein D (ApoD), an androgen inducible protein[13,15], in several digestive tumors[16]. Likewise, its tumoral expression in HCC, has been recently found to be significantly lower, than in non-tumour liver tissue[17]. In addition, a low ApoD expression has been related to the presence of undifferentiated HCC and a less poor prognosis. Thus based on these findings, it could be speculated that ApoD expression is a possible biochemical marker of androgen action in HCC, and therefore, it would have a clinical interest in order to select patients as candidates for specific hormonal therapeutic strategies.

Having all these facts to consider, we believe it could be important to identify and characterize the molecular abnormalities of several hormonal receptors and ApoD and to evaluate their potential clinical significance in surgically treated HCC, in order to develop future clinical trials based on different hormonal approaches[18].

MATERIALS AND METHODS

Patient selection

The study population consisted of 31 patients diagnosed with HCC during a 13 years period (1989-2002), before starting a program of liver transplantation in the same center and followed since then, at the University Hospital Central of Asturias in Oviedo, Spain.

All cases were diagnosed on a clinical basis, with imaging and pathological confirmation of the tumor, and presented with nodular limited disease and associated cirrhosis stage A (Child-Pugh criteria). In addition, all patients were considered degree A, according to the Barcelona Clinic Liver Cancer (BCLC) staging system[19].

When feasible, tumors were removed by surgical resection (n = 24) and when not, liver transplantation was performed (n = 7). The selection criteria for the intervention were as follows: (1) Patients underwent surgical resection if they had solitary tumors, of 5 cm or less in size, with normal bilirubin serum levels, and no signs of portal hypertension. (2) Patients were treated by liver transplantation if they had multiple tumors ($\leqslant$ 3 nodes of $\leqslant$ 3 cm in size), no significant comorbidity, or if they were not good candidates for liver resection. We did not include patients with extended disease nor associated liver failure.

During the follow-up period, two patients were treated with percutaneous ethanol injection (PEI) for local relapse and five cases underwent Trans-Arterial Chemo-Embolization (TACE), for intrahepatic tumor spread. Main basal characteristics of the patient cohort, are shown in Table 1. The actuarial survival of patients included in the present study was performed during the follow-up period, ending on December 31, 2006.

Study evaluations

Age, sex, diagnosis, and etiology of cirrhosis were assessed. The degree of liver insufficiency was evaluated according to Child-Pugh's classification[20].

Blood samples were obtained for cell blood counts (CBC), coagulation study, renal evaluation, serum albumin levels, liver function tests (LFTs), serum viral hepatitis markers and sequential AFP determinations.

Table 1 Clinico-pathological and biological parameters of patients

Characteristics	*n* (%)	Deaths, *n* (%)	RR (95% CI)
Males	26 (83.9)	14 (53.8)	1.14 (0.36-3.57)
Age < 65 yr	21 (67.7)	12 (57.1)	1 (0.37-2.70)
Cirrhosis	29 (93.5)	18 (62)	21.85 (0-187.50)
HCV (+)	16 (51.6)	10 (62.5)	0.80 (0.31-2.06)
Alcohol etiology	13 (42.0)	11 (84.6)	0.62 (0.23-1.62)
Uninodular	25 (80.6)	15 (60)	1.33 (0.37-4.74)
Size $\leqslant$ median mm	21 (67.7)	10 (47.6)	4.73 (1.61-13.89)[a]
Alpha-fetoprotein $\geqslant$ 20 (ng/mL)	12 (38.7)	8 (66.6)	1.46 (0.57-3.71)
Histopathology HCC-T	28 (90.3)	16 (55.1)	1.35 (0.30-5.95)
AR (+)	21 (67.7)	10 (47.6)	0.62 (0.25-1.68)
ER (+)	16 (51.6)	10 (62.5)	1.05 (0.41-2.67)
PgR (+)	26 (83.9)	13 (50)	0.26 (0.08-0.85)[a]
ApoD (+)	12 (38.7)	8 (66.6)	1.47 (0.57-3.77)

[a]P < 0.05. HCC-T: HCC trabecular.

Patient follow-up was planned every 3 mo until death; at each follow-up visit, a clinical examination was performed and hematological and biochemical parameters were obtained, including α-fetoprotein levels. Abdominal ultrasound and/or CT were done when needed for detection of local relapse. In addition, the Child-Pugh´s score and WHO performance status, were also assessed in each visit.

Tissue microarrays (TMAs) and immunohistochemistry

Routinely fixed (overnight in 10% buffered formalin), paraffin-embedded tumor samples stored in our Pathology Laboratory Files, were used in this study. Histopathologically representative tumor areas were defined on hematoxylin and eosin-stained sections and marked on the slide. TMA blocks were obtained by punching a tissue cylinder (core) with a diameter of 1.5 mm through a histologically representative area of each 'donor' tumor block, which was then inserted into an empty 'recipient' tissue array paraffin block using a manual tissue arrayer (Beecher Instruments, Sun Prairie, Wisconsin, USA) as described elsewhere[21]. Collection of tissue cores was carried out under highly controlled conditions. Areas of non-necrotic tumor tissue were selected for arraying by two experienced pathologists (LO. G and FF). Two cores were used for each case. One tissue array block was prepared from the 31 tumor samples available, each one containing 62 tumor samples, as well as internal controls including four liver tissue samples obtained from recently procured necropsy material from two individuals who died of non-liver related disease.

One composite high-density TMA block was designed and serial 5-μm sections were consecutively cut with a microtome (Leyka Mikrosysteme Vertrieb GmbH, Bensheim, Germany) and transferred to adhesive-coated slides. One section from each tissue array block was stained with H&E, and these slides were then reviewed to confirm that the sample was representative of the original tumor. The other sections were incubated with anti-AR clone AR 441 (Dako, Glostrup, Denmark) at a dilution of 1/50, the anti-ER clone 1D5 (Dako), the anti-PR clone PgR 636 (Dako), and the anti-ApoD clone 8CD6 (Signet,

Dedham, Massachusetts, USA) at a dilution of 1/100. All dilutions were made in antibody diluent, (Dako) for 30 min at room temperature.

Tissue sections were deparaffinized in xylene, and then rehydrated in graded concentrations of ethyl alcohol (100%, 96%, 80%, 70%, then water). To enhance antigen retrieval for all antibodies, TMA sections were microwave-treated (H2800 Microwave Processor, EBSciences, East Granby, Connecticut, USA) in citrate buffer (Target Retrieval Solution, Dako) at 99℃ for 15 min. Endogenous peroxidase activity was blocked by incubating the slides in peroxidase-blocking solution (Dako ChemMate™) for 5 min. The EnVision™ Detection, and the peroxidase/DAB kits (Dako) were used as the staining detection system. The sections were counterstained with hematoxylin, dehydrated with ethanol, and permanently coverslipped. Specificity of the staining was determined using controls that involved incubation of tissue sections with antibody diluent (Dako) alone. In these cases there was no significant staining.

TMA analysis

For each antibody preparation studied, the location of immunoreactivity, percentage of stained cells and intensity were determined. All the cases were semiquantified for each protein-stained area. An image analysis system composed of the Olympus BX51 microscope and soft analysis (AnalySIS®, Soft Imaging System, Münster, Germany) was used as follows: tumor sections were stained with antibodies according to the method explained above and counterstained with Hematoxilin. There are different optical thresholds for both stains. Each core was scanned with a 400 X power objective in two fields per core. Fields were chosen searching for the protein-stained areas. The computer program selects and traces a line around antibody-stained areas (higher optical threshold: red spots), with the remaining, non-stained areas (hematoxylin-eosin stained tissue with lower optical threshold) standing out as a blue background. All fields have an area ratio of stained (red) versus non-stained areas (blue). A final area ratio was obtained after averaging two fields. To evaluate immunostaining intensity we used a numeric score ranging from 0 to 3, reflecting the intensity as follows: 0, absence; 1, weak; 2, moderate; and 3, intense staining.

Using an Excel spreadsheet (Microsoft Corporation, Redmond, Washington, USA), the mean score was obtained by multiplying the intensity score (I) by the percentage of stained cells (PSC) and the results were added together (total score: I × PSC). This overall score was then averaged with the number of cores that were done for each patient. If there was no staining tumor in a particular core, then no score was given. In addition, for each tumor, the mean score of two core biopsies was calculated.

A case was defined as positive if at least 10% of the tumor cells were weakly stained, or at least 1% of the tumor cells showed moderate or intense staining. Furthermore, whole-tissue sections from tumor blocks from a subset of ten cases were compared with the corresponding TMA discs, regarding each protein expression. Those cases were randomly selected, and the obtained clinicopathological data were very similar to those from the whole series. Each whole-tissue section was scanned with a 400 × power lens in ten different fields. Fields were selected by searching for the protein-stained areas, such as it was described above.

Statistical analysis

After analyzing the distribution of the score values for immunohistochemical staining (I × PSC) for each protein by the Kolmogorov-Smirnov test, non-parametric rank methods were used to analyze the data. Immunostaining values for each protein were expressed as the median (range). Patients were subdivided into different groups, based on their clinical and pathological parameters. Comparison of immunostaining values between groups was made with the Mann-Whitney or Kruskal-Wallis tests. Correlations between continuous variables were calculated by the Spearman test. Differences in percentages were calculated with the Chi-square test. Survival curves were calculated by the Kaplan-Meier method and compared with the log-rank test. Cox's regression model was used to examine interactions of different prognostic factors in a multivariate analysis. In the multivariate analysis, we included only parameters that achieved statistical significance for relapse-free survival in the univariate analysis. Survival rates are presented with their 95% confidence intervals (95% CI). Statistical tests were two-sided at a 5% probability level ($P < 0.05$). A SPSS 11.5 program (SPSS Inc, Chicago, Illinois, USA) was used for the calculations.

RESULTS

A total of 260 determinations in different tumor specimens from 31 patients with resectable HCC and controls, were performed on TMAs. Minimal internal variance of score data between duplicate tissue cores from the same patient was detected in the tissue arrays, showing a high agreement ($r > 0.95$ and $^{d}P < 0.0001$, for each protein). In the validation study, there was a total concordance in the global expression as well as in the intensity of immunostaining for each protein, between TMA cases and the corresponding whole-tissue sections. In addition, there was a highly significant correlation in the immunostaining scores between these two paired sets ($r > 0.90$ and $^{d}P < 0.0001$, for each protein).

Figure 1 shows examples of tissue microarrays with immunostaining for each protein evaluated in the study. As expected, AR, ER and PgR immunostaining were of nuclear localization; whereas ApoD-positive staining was localized in the cytoplasm of the malignant cells. A total of 21 tumors stained positively (67.7%) for AR, 16 (51.6%) for ER, 26 (83.8%) for PgR, and 12 (38.7%) for ApoD. In addition, there was a wide variability in the immunostaining score values for each protein in the positive cases: AR median (range), 11.5 (11.5-229.5); ER, 11.1 (8.5-65); PgR, 14.2 (4-61); and ApoD, 37.7 (13.8-81.1). We analyzed the possible relationship between the expression of the studied proteins and some patients' clinicopathological characteristics such as gender, age, liver cirrhosis, HCV infection, alcohol etiology, Child-Pugh's score, portal vein thrombosis, tumor multiplicity and size, alpha-fetoprotein

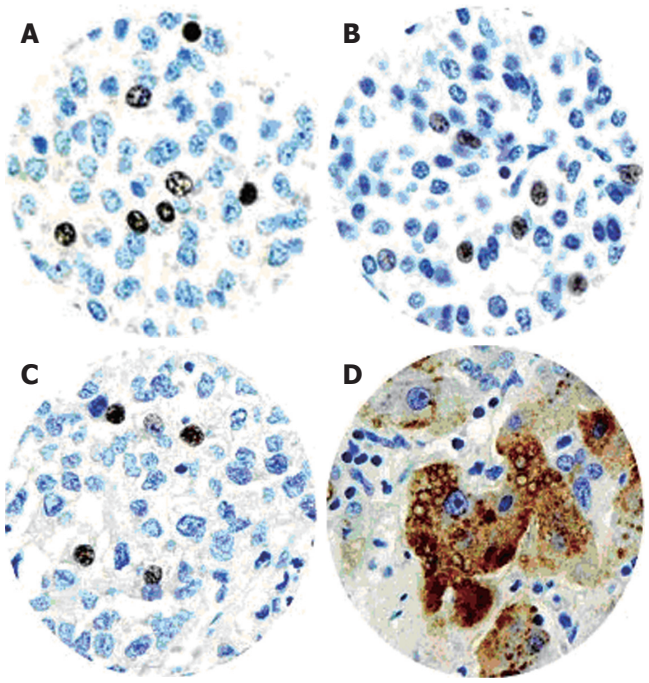

Figure 1 Expression of proteins studied by Immunohistochemistry on TMA. Immunohistochemical staining of a tumor core. **A**: AR; **B**: ER; **C**: PgR; **D**: ApoD (x 400).

serum levels and tumor histology (Table 1). Thus, we found that ER values correlated negatively with alcohol etiology ($^aP < 0.05$), and positively with both PgR-positive ($^aP < 0.05$) and AR-positive status ($^aP < 0.05$); whereas PgR values correlated positively with HVC infection ($^bP < 0.01$) and AR-positive status ($^aP < 0.05$). However, statistical analysis revealed that Apo D immunostaining values did not correlate with any of those clinico-pathological or biological characteristics (Tables 2 and 3).

We also analyzed the association between the status for each protein and the overall patient survival. We have only found a significant association between PgR status and outcome ($P = 0.0169$). Patients whose tumors showed a negative immunostaining for PgR had a poorer overall survival (Figure 2). We have also looked into the possible prognostic value of the median score value chosen as a cut-off point for each protein. But, significant associations with overall survival were not found (data not shown). Multivariate analysis according to the Cox model demonstrated that negative PgR status ($P = 0.002$), was the only parameter significantly and independently associated with overall survival.

Table 2 Relationship between AR and ER Immunostaining values and Clinico-Pathological Characteristics

Characteristics	Androgen receptors (AR)		Estrogen receptors (ER)	
	n (%)	Score values median (range)	*n* (%)	Score values median (range)
Males	18 (69.2)	4.1 (0-229.5)	12 (46.2)	8.5 (0-65)
Age < 65 yr	15 (71.4)	6.7 (0-52)	10 (47.6)	8.5 (0-65)
Cirrhosis	19 (65.5)	4.2 (0-229.5)	16 (55.2)	8.5 (0-65)
VHC (+)	11 (68.8)	4.2 (0-229.5)	11 (68.8)	8.75 (0-65)
Alcohol etiology	7 (53.8)	3.7 (0-52)	3 (23.1)	0 (0-65)
Uninodular	16 (64)	4.2 (0-229.5)	13 (52)	8.5 (0-65)
Tumor size				
< median	13 (61.9)	4 (0-229.5)	12 (57.1)	8.5 (0-65)
⩾ median	8 (80)	5.75 (0-16)	4 (40)	8.7 (0-15.75)
Alfa-fetoprotein (ng/mL)				
< 20	12 (63.2)	4 (0-229.5)	8 (42.1)	8.5 (0-65)
⩾ 20	9 (75)	5.7 (0-31)	8 (66.7)	8.7 (0-16)
Histopathology				
HCC-T	20 (71.4)	4.5 (0-229.5)	15 (53.6)	8.5 (0-65)
Non HCC-T	1 (33.3)	1 (0-26.25)	1 (33.3)	0 (0-20.5)
Death				
No	11 (84.6)	12 (0-229.5)	6 (46.2)	9 (0-65)
Yes	10 (55.6)	4 (0-28.75)	10 (55.6)	8.5 (0-20.5)
Androgen receptors				
Negative	-	-	3 (30)	0 (0-20.5)[a]
Positive			13 (61.9)	9 (0-65)
Estrogen receptors				
Negative	8 (53.3)	3.2 (0-29)	-	-
Positive	13 (81.3)	8.4 (0-229.5)		
Progesterone receptors				
Negative	0 (0)[c]	1.7 (0-4.2)[a]	1 (20)	0 (0-8.5)[a]
Positive	21 (80.8)	8.4 (0-229.5)	15 (57.7)	9 (0-65)
Apo D				
Negative	13 (68.4)	4.2 (0-52)	10 (52.6)	8.5 (0-65)
Positive	8 (66.7)	4.2 (0-229.5)	6 (50)	8.5 (0-65)

$^aP < 0.05$, $^cP < 0.005$. HCC-T: HCC trabecular.

DISCUSSION

This is to our knowledge, the first study designed to analyze the combined expression of AR, ER, PgR, and ApoD in human HCCs by applying TMA technology, which has allowed us to process a large number of tissue specimens from HCC patients for a wide range of protein determinations, and to associate staining results with various clinicopathological parameters. Thus, this technology offers the opportunity to integrate different biological aspects of the tumor into the morphological context of HCCs. Our results demonstrate a wide variability in the immunohistochemical values for steroid receptors and ApoD among HCCs, supporting the concept that HCC is a heterogeneous disease. In addition, we believe this is the first study reporting that ER expression by liver carcinomas correlates negatively with alcohol etiology, whereas PgR expression correlates positively with VHC infection, as well as, with a better prognosis. Thus, these results suggest both a biological and a clinical interest for steroid receptor determination in HCCs.

Our data are in accordance with other investigations indicating that some, but not all, specimens of HCCs have elevated AR, ER, PgR[9,11,22,23], indicating that determination of steroid receptor content might be useful prior to initiation of certain hormone-blocking therapies. However, the available studies on tamoxifen treatment of HCC have produced conflicting results in that both, a survival benefit in patients with advanced HCC[24,26] as well as a worse survival[27,32] have been reported. In a recent study, tamoxifen did not improve the survival of patients with advanced HCC. But, there was a suggestion that patients without major hepatic insufficiency seemed to have some survival benefit[12]. Lack of tamoxifen efficacy in terms of tumor growth and survival could be ascribed either to a low expression of ER in HCC[33] or to the expression

Table 3 Relationship between PgR and Apo D Immunostaining values and clinico-pathological characteristics

Characteristics	Progesterone receptors (PgR)		Apolipoprotein-D (Apo D)	
	n (%)	Score (median-range)	*n* (%)	Score (median-range)
Males	22 (84.6)	13.7 (0-61)	10 (28.5)	0 (0-81.1)
Age < 65 yr	17 (81)	9.7 (0-61)	7 (33.3)	0 (0-69.5)
Cirrhosis	24 (82.8)	14 (0-61)	11 (37.9)	0 (0-81.1)
HCV (+)	15 (93.8)	15.9 (3-61)	8 (50)	11 (0-81.11)
Alcohol etiology	11 (84.6)	9.7 (0-25)	5 (38.5%)	0 (0-80.4)
Uninodular	20 (80)	13.7 (0-61)	11 (44)	0 (0-81.1)
Size < median	17 (81)	13.7 (0-61)	6 (28.6)	0 (0-80.4)
Alfa-fetoprotein ⩾ 20 (ng/mL)	11 (91.7)	14 (5.7-61)	3 (25)	0 (0-80.4)
Histopathology				
HCC-T	23 (82.1)	13.7 (0-61)	11 (39.3)	0 (0-81.1)
Death rate	13 (72.2)	11.75 (0-61)	8 (44.4)	0 (0-81.1)
Androgen receptors				
Negative	5 (50)[c]	9.6 (0-20)[a]	4 (40)	0 (0-80.4)
Positive	21 (100)	14.2 (4-61)	8 (38.1)	0 (0-81.1)
Estrogen receptors				
Negative	11 (73.3)	8 (0-34)[a]	6 (40)	0 (0-81.1)
Positive	15 (93.8)	17.2 (3-61)	6 (37.5)	0 (0-80.4)
Pg receptors				
Negative	-	-	2 (40)	0 (0-24.4)
Positive			10 (38.5)	0 (0-81.1)
ApoD				
Negative	16 (84.2)	13.7 (0-61)	-	-
Positive	10 (83.3)	13.8 (3-34)		

[a]$P < 0.05$, [c]$P < 0.005$. HCC-T: HCC trabecular.

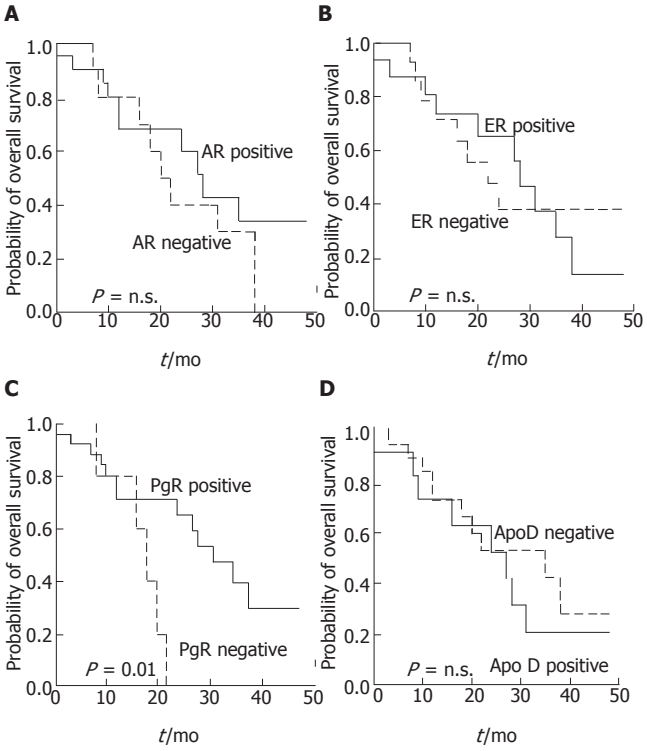

Figure 2 Overall Survival as a function of the AR (**A**), ER (**B**), PgR (**C**) and ApoD (**D**) tumoral expressions.

of mutated ERs, although it has also been suggested that the effect of tamoxifen treatment is not affected by the expression of ER[31]. Nevertheless, the consideration that PgR is an estrogen-inducible protein, led us to think that this steroid receptor could be a potential marker for the functional stage of ERs in HCC, in order to select patient candidates for anti-estrogen therapy. In addition, our study demonstrates that PgR expression has a prognostic implication in patients with resected HCC as a factor associated with an improved prognosis, a clinical finding previously demonstrated in breast cancer[34,35]. We consider that this result could be of great interest considering our reported positive association between PgR expression and HCV infection, which represents an increasing etiology of HCC in our patient's population[2].

Previous studies showed that AR expression was associated with intrahepatic recurrence in HCC[33]. In other studies, AR expression was also associated with a small tumor size, but not with a higher rate of recurrence[23]. However, we found no significant relationships between AR expression and clinicopathological parameters or clinical outcome in our population of patients with resected HCC. Although there are few data on the effect of anti-androgens in patients diagnosed with HCC, it has been reported that anti-androgen therapy might have some benefit in patients with androgen-positive tumors[22]. Likewise, the results of a multicenter trial with anti-androgenic drugs, in male patients with HCC were recently reported, although the study was interrupted because of intolerable digestive side effects[36]. Nevertheless, further studies are necessary to assess whether AR status could be used as a marker to more accurately select patients as candidates for further anti-androgenic therapy.

In the present study, we have also investigated ApoD expression by HCCs, an androgen-inducible protein in both prostate and breast cancer cells[13,15], in consideration to the recent report from Utsunomiya *et al*[17], showing that a low ApoD expression significantly correlated with less-differentiated HCCs and with a worse prognosis. However, we did not find any relationship between ApoD expression and the clinico-pathological parameters evaluated or clinical outcome. This may be due to the small series of patients included in the present study or to the different criteria used for patient selection. Moreover, we found no significant relationship between the lipoprotein expression and the hormonal receptor status. Nevertheless, further studies are also necessary in order to assess the possible value of ApoD as a biological marker of androgen response and/or of other hormonal pathways in HCC. Thus, although ApoD is an androgen-regulated protein, we also have to consider that other hormonal steroids and other substances such as steroids, retinoic acid and 1.25-dihydroxy vitamin D_3, that also induce ApoD expression, may be involved in its regulation in human tumors[14,37,38], mostly if we consider that both retinoid and steroid receptors have also been detected in HCC[39,40] and that available preliminary data have shown that retinoids are of clinical benefit as cancer chemotherapeutic agents in advanced HCC[41].

In summary, our results show a clinical value of the steroid receptor status in HCC, and open the opportunity to design further studies pointing towards the development of new endocrine strategies in patients affected by this increasing tumoral disease.

REFERENCES

1 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108
2 **El-Serag HB**, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. *N Engl J Med* 1999; **340**: 745-750
3 **Poon RT**, Ng IO, Fan ST, Lai EC, Lo CM, Liu CL, Wong J. Clinicopathologic features of long-term survivors and disease-free survivors after resection of hepatocellular carcinoma: a study of a prospective cohort. *J Clin Oncol* 2001; **19**: 3037-3044
4 **Francavilla A**, Polimeno L, DiLeo A, Barone M, Ove P, Coetzee M, Eagon P, Makowka L, Ambrosino G, Mazzaferro V. The effect of estrogen and tamoxifen on hepatocyte proliferation in vivo and in vitro. *Hepatology* 1989; **9**: 614-620
5 **Clavière C**, Bronowicki JP, Hudziak H, Bigard MA, Gaucher P. Role of sex steroids and their receptors in the pathophysiology of hepatocellular carcinoma. *Gastroenterol Clin Biol* 1998; **22**: 73-86
6 **Villa E**, Grottola A, Colantoni A, De Maria N, Buttafoco P, Ferretti I, Manenti F. Hepatocellular carcinoma: role of estrogen receptors in the liver. *Ann N Y Acad Sci* 2002; **963**: 37-45
7 **Eagon PK**, Elm MS, Epley MJ, Shinozuka H, Rao KN. Sex steroid metabolism and receptor status in hepatic hyperplasia and cancer in rats. *Gastroenterology* 1996; **110**: 1199-1207
8 **Nagasue N**, Yu L, Yukaya H, Kohno H, Nakamura T. Androgen and oestrogen receptors in hepatocellular carcinoma and surrounding liver parenchyma: impact on intrahepatic recurrence after hepatic resection. *Br J Surg* 1995; **82**: 542-547
9 **Nagasue N**, Kohno H, Yamanoi A, Kimoto T, Chang YC, Nakamura T. Progesterone receptor in hepatocellular carcinoma. Correlation with androgen and estrogen receptors. *Cancer* 1991; **67**: 2501-2505
10 **Jonas S**, Bechstein WO, Heinze T, Kling N, Lobeck H, Tullius SG, Steinmueller T, Neuhaus P. Female sex hormone receptor status in advanced hepatocellular carcinoma and outcome after surgical resection. *Surgery* 1997; **121**: 456-461
11 **Ng IO**, Ng M, Fan ST. Better survival in women with resected hepatocellular carcinoma is not related to tumor proliferation or expression of hormone receptors. *Am J Gastroenterol* 1997; **92**: 1355-1358
12 **Barbare JC**, Bouché O, Bonnetain F, Raoul JL, Rougier P, Abergel A, Boige V, Denis B, Blanchi A, Pariente A, Milan C, Bedenne L. Randomized controlled trial of tamoxifen in advanced hepatocellular carcinoma. *J Clin Oncol* 2005; **23**: 4338-4346
13 **Simard J**, Dauvois S, Haagensen DE, Lévesque C, Mérand Y, Labrie F. Regulation of progesterone-binding breast cyst protein GCDFP-24 secretion by estrogens and androgens in human breast cancer cells: a new marker of steroid action in breast cancer. *Endocrinology* 1990; **126**: 3223-3231
14 **Simard J**, de Launoit Y, Haagensen DE, Labrie F. Additive stimulatory action of glucocorticoids and androgens on basal and estrogen-repressed apolipoprotein-D messenger ribonucleic acid levels and secretion in human breast cancer cells. *Endocrinology* 1992; **130**: 1115-1121
15 **Sugimoto K**, Simard J, Haagensen DE, Labrie F. Inverse relationships between cell proliferation and basal or androgen-stimulated apolipoprotein D secretion in LNCaP human prostate cancer cells. *J Steroid Biochem Mol Biol* 1994; **51**: 167-174
16 **Yamashita K**, Upadhyay S, Osada M, Hoque MO, Xiao Y, Mori M, Sato F, Meltzer SJ, Sidransky D. Pharmacologic unmasking of epigenetically silenced tumor suppressor genes in esophageal squamous cell carcinoma. *Cancer Cell* 2002; **2**: 485-495
17 **Utsunomiya T**, Ogawa K, Yoshinaga K, Ohta M, Yamashita K, Mimori K, Inoue H, Ezaki T, Yoshikawa Y, Mori M. Clinicopathologic and prognostic values of apolipoprotein D alterations in hepatocellular carcinoma. *Int J Cancer* 2005; **116**: 105-109
18 **Thomas MB**, Abbruzzese JL. Opportunities for targeted therapies in hepatocellular carcinoma. *J Clin Oncol* 2005; **23**: 8093-8108
19 **Llovet JM**, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999; **19**: 329-338
20 **Pugh RN**, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973; **60**: 646-649
21 **Parker RL**, Huntsman DG, Lesack DW, Cupples JB, Grant DR, Akbari M, Gilks CB. Assessment of interlaboratory variation in the immunohistochemical determination of estrogen receptor status using a breast cancer tissue microarray. *Am J Clin Pathol* 2002; **117**: 723-728
22 **Boix L**, Bruix J, Castells A, Fuster J, Bru C, Visa J, Rivera F, Rodes J. Sex hormone receptors in hepatocellular carcinoma. Is there a rationale for hormonal treatment? *J Hepatol* 1993; **17**: 187-191
23 **Boix L**, Castells A, Bruix J, Solé M, Brú C, Fuster J, Rivera F, Rodés J. Androgen receptors in hepatocellular carcinoma and surrounding liver: relationship with tumor size and recurrence rate after surgical resection. *J Hepatol* 1995; **22**: 616-622
24 **Farinati F**, Salvagnini M, de Maria N, Fornasiero A, Chiaramonte M, Rossaro L, Naccarato R. Unresectable hepatocellular carcinoma: a prospective controlled trial with tamoxifen. *J Hepatol* 1990; **11**: 297-301
25 **Martínez Cerezo FJ**, Tomás A, Donoso L, Enríquez J, Guarner C, Balanzó J, Martínez Nogueras A, Vilardell F. Controlled trial of tamoxifen in patients with advanced hepatocellular carcinoma. *J Hepatol* 1994; **20**: 702-706
26 **Elba S**, Buongiorno GP, Caruso ML, Noviello MR, Manghisi OG. Main characteristics of hepatocellular carcinoma and cirrhosis and therapeutic approaches. *Curr Pharm Des* 2002; **8**: 1007-1011
27 **Castells A**, Bruix J, Brú C, Ayuso C, Roca M, Boix L, Vilana R, Rodés J. Treatment of hepatocellular carcinoma with tamoxifen: a double-blind placebo-controlled trial in 120 patients. *Gastroenterology* 1995; **109**: 917-922
28 **Manesis EK**, Giannoulis G, Zoumboulis P, Vafiadou I, Hadziyannis SJ. Treatment of hepatocellular carcinoma with combined suppression and inhibition of sex hormones: a randomized, controlled trial. *Hepatology* 1995; **21**: 1535-1542
29 **Tamoxifen in treatment of hepatocellular carcinoma: a randomised controlled trial**. CLIP Group Cancer of the Liver Italian Programme. *Lancet* 1998; **352**: 17-20
30 **Riestra S**, Rodriguez M, Delgado M, Suárez A, González N, de la Mata M, Diaz G, Miño-Fugarolas G, Rodrigo L. Tamoxifen does not improve survival of patients with advanced hepatocellular carcinoma. *J Clin Gastroenterol* 1998; **26**: 200-203
31 **Liu CL**, Fan ST, Ng IO, Lo CM, Poon RT, Wong J. Treatment of advanced hepatocellular carcinoma with tamoxifen and the correlation with expression of hormone receptors: a prospective randomized study. *Am J Gastroenterol* 2000; **95**: 218-222
32 **Chow PK**, Tai BC, Tan CK, Machin D, Win KM, Johnson PJ, Soo KC. High-dose tamoxifen in the treatment of inoperable hepatocellular carcinoma: A multicenter randomized controlled trial. *Hepatology* 2002; **36**: 1221-1226
33 **Grazi GL**, Cescon M, Ravaioli M, Ercolani G, Gardini A, Del Gaudio M, Vetrone G, Cavallari A. Liver resection for hepatocellular carcinoma in cirrhotics and noncirrhotics. Evaluation of clinicopathologic features and comparison of risk factors for long-term survival and tumour recurrence in a single centre. *Aliment Pharmacol Ther* 2003; **17** Suppl 2: 119-129
34 **Stendahl M**, Rydén L, Nordenskjöld B, Jönsson PE, Landberg G, Jirström K. High progesterone receptor expression correlates to the effect of adjuvant tamoxifen in premenopausal breast cancer patients. *Clin Cancer Res* 2006; **12**: 4614-4618
35 **Punglia RS**, Kuntz KM, Winer EP, Weeks JC, Burstein HJ. The impact of tumor progesterone receptor status on optimal adjuvant endocrine therapy for postmenopausal patients with early-stage breast cancer: a decision analysis. *Cancer* 2006; **106**: 2576-2582
36 **Randomized trial of leuprorelin and flutamide in male patients with hepatocellular carcinoma treated with**

tamoxifen. *Hepatology* 2004; **40**: 1361-1369
37 **López-Boado YS**, Tolivia J, López-Otín C. Apolipoprotein D gene induction by retinoic acid is concomitant with growth arrest and cell differentiation in human breast cancer cells. *J Biol Chem* 1994; **269**: 26871-26878
38 **López-Boado YS**, Díez-Itza I, Tolivia J, López-Otín C. Glucocorticoids and androgens up-regulate the Zn-alpha 2-glycoprotein messenger RNA in human breast cancer cells. *Breast Cancer Res Treat* 1994; **29**: 247-258
39 **P'eng FK**, Lui WY, Chang TJ, Kao HL, Wu LH, Liu TY, Chi CW. Glucocorticoid receptors in hepatocellular carcinoma and adjacent liver tissue. *Cancer* 1988; **62**: 2134-2138
40 **Sano K**, Takayama T, Murakami K, Saiki I, Makuuchi M. Overexpression of retinoic acid receptor alpha in hepatocellular carcinoma. *Clin Cancer Res* 2003; **9**: 3679-3683
41 **Clerici C**, Castellani D, Russo G, Fiorucci S, Sabatino G, Giuliano V, Gentili G, Morelli O, Raffo P, Baldoni M, Morelli A, Toma S. Treatment with all-trans retinoic acid plus tamoxifen and vitamin E in advanced hepatocellular carcinoma. *Anticancer Res* 2004; **24**: 1255-1260

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