
Androgen Receptor in Hepatocellular Carcinoma as a Prognostic Factor After Hepatic Resection

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Androgen receptors (AR) in the cytosol of hepatocellular carcinoma (HCC) were assayed in 45 unselected patients in whom radical hepatic resection was performed. Thirty-one patients had detectable amounts of ARs in tumors, ranging from 2.3 to 82.6 fmol/mg protein with the dissociation constants (Kd) of $4.1 - 30.9 \times 10^{-10}$ M. The receptor was not found in the remaining 14 cases. AR negative HCCs were significantly more common among women and nonalcoholic patients. Otherwise, there were no significant difference in the clinicopathologic background between patients with AR positive HCCs and those with AR negative tumors. Three patients died of liver failure in the former group, whereas two died in the latter; one patient died of liver failure and the other died of pneumonia (results were not statistically significant). Excluding those five operative deaths, the recurrence rates were 67.9% in the group of patients with AR positive HCCs and 33.3% in the group of patients with AR negative tumors ($0.1 < p < 0.05$). The 5-year survival rate was significantly better ($p < 0.05$) in patients with AR negative HCCs (62.2%) than in those with the positive tumors (17.3%). In light of the current results and previous experimental works by others, it is likely that testosterone enhance the growth and invasiveness of human HCC, which is mediated by AR in the tumor.

HEPATOCELLULAR CARCINOMA (HCC) is one of the most malignant tumors in humans.¹ Due to a high incidence of associated liver cirrhosis or early spread in the liver, its resectability is generally low.²⁻⁴ Nevertheless, hepatic resection is at present the most reliable and acknowledged method for cure, although liver transplantation may be more ideal in selected patients.⁵ Several recent investigations have reported prognostic factors after resection of HCC.⁶⁻⁹ According to these reports, the patient's prognosis can be considered poor if the tumor is large, invasive, or nonencapsulated, or if the patient has symptoms due to HCC, elevated preoperative

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serum alpha-fetoprotein (AFP) level, or inadequate surgical margin.

During recent years, we have investigated sex hormone receptor profiles in HCC and the surrounding liver, and suggested a possibility that this tumor is androgen-dependent¹⁰⁻¹². In the current investigation, we have analyzed the recurrence rate and long survival rate after radical hepatic resection between the patients with androgen receptor (AR) positive HCCs and those with AR negative tumors.

Materials and Methods

During the past 6.5 years, ARs were assayed for primary hepatocellular carcinoma (HCC) in 45 unselected patients in whom radical hepatic resection was performed. ARs in the tumor were detected in 31 patients but not in the remaining 14. Long-term survival and tumor recurrence rates were compared between those patients with AR positive and negative HCCs. The mean follow-up times were 36.2 ± 18.4 months in the AR positive group and 31.4 ± 18.0 months in the AR negative group.

There was no significant difference in age distribution. AR negative HCCs were seen more frequently in women than in men ($p < 0.02$). Alcohol abuse was noted in 15 patients of the AR positive group but in none of the AR negative group ($p < 0.01$). There were no significant differences between the two study groups regarding the extent of underlying liver disease, positive rate of hepatitis B virus, and serum AFP level (Table 1). Table 2 shows the results of preoperative liver tests. There were no substantial differences between the two groups. Due to a high inci-

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TABLE 1. Preoperative Clinical Background

Data	AR-positive (No. of Patients)	AR-negative (No. of Patients)
Total number of patients	31	14
Age (years)*	59.4 ± 8.8	59.2 ± 8.4
Men/women	25/4‡	6/8‡
Duration of liver disease (years)*	6.8 ± 6.3	8.1 ± 6.6
Alcohol abuse†		
Yes	15§	0§
No	16	14
Child's class		
A	17	7
B	11	1
C	3	3
Hepatitis B virus		
HBs-antigen	17	7
Anti-HBs	14	1
Anti-HBc	25	7
Serum AFP (ng/ml)		
Less than 20	7	4
20-1000	20	8
1000-10,000	3	0
More than 10,000	1	2

* Mean ± SD

† Those who consume more than 50 g of alcohol every day for more than 5 years.

‡ $p < 0.02$.§ $p < 0.01$.

dence of associated liver cirrhosis, atypical minor hepatic resections were performed in most patients. No statistical difference was found in the extent of liver resection between the two groups (Table 3). Histopathologic data of the resected specimens are shown in Table 4. No significant differences in tumor size, histologic type of HCC and associated liver diseases were observed between the two study groups.

Postoperative Follow-up

Twenty-four patients received postoperative chemotherapy. The incidence and type of chemotherapy did not differ between the two groups. All patients, except for those who died during operation, were evaluated with computed tomography, ultrasonography, and, if necessary, angiography just before discharge from hospital. They were all proven to have no residual tumors in the liver and no distant metastases. After discharge from the hospital, the patients were followed up at a 2-week interval during the first 6 months and monthly thereafter. Imaging diagnosis was repeated every 3 months. When tumor recurrence was suspected, the patient was hospitalized for angiography. Whenever the recurrent tumor was resectable, second hepatic resection was performed. Otherwise, chemotherapy with or without embolization of the hepatic artery was performed.

TABLE 2. Preoperative Liver Tests

Data	AR-positive (No. of Patients)	AR-negative (No. of Patients)
AST (IU/ml)*	99 ± 45	78 ± 42
ALT (IU/ml)†	78 ± 38	76 ± 66
LDH (IU/ml)	349 ± 91	346 ± 84
Bilirubin (mg/100 ml)	1.0 ± 0.5	0.9 ± 0.4
Albumin (g/100 ml)	3.6 ± 0.5	3.6 ± 0.7
Cholesterol (mg/100 ml)	142 ± 33	153 ± 30
BSP (30 minutes)‡	26.8 ± 15.3	32.7 ± 12.7
ICG (15 minutes)§	24.1 ± 13.7	26.1 ± 13.9

* Aspartate aminotransferase (normal, 0-37).

† Alanine aminotransferase (normal, 3-32).

‡ Bromosulphalein retention rate at 30 minutes (normal, 0-10%).

§ Indocyanine green retention rate at 15 minutes (normal, 0-10%).

AR Assay

In all cases, specimens for analysis were obtained at the time of operation. The tumor was frozen in liquid nitrogen immediately after removal and was stored at -80 C until the receptor assays could be performed. The chemicals and the method of cytosol preparation are described elsewhere.¹⁰ Aliquots of cytosol (0.15 ml) were incubated with increasing concentrations of ³H-R1881 (0.25-4 nM) and 0.125-2 μM triamcinolone with or without 4 μM radioinert R1881 for 16-18 hours. After the incubation, 50 μM dextran-coated solution (2.5% activated charcoal Norit A and 0.025% dextran T-70 in TEGT [10 mM TRIS-HCl, 1.5 mε EDTA, 30% (u/v) glycerol, and 1 mM thioglycerol] buffer) was added to the tubes, which were incubated for 20 minutes and then centrifuged for 15 minutes at 1500 g. The radioactivity in the supernatant was measured by liquid scintillation counting. The data were analyzed by the method of Scatchard¹³ after subtraction of nonspecific androgen binding.

Statistical Methods

Statistical comparisons for significance were made with the use of unpaired Student's t-test and the chi square test with a single degree of freedom; p values of less than 0.05 were considered statistically significant. Cumulative survival rates were obtained by means of the Kaplan-Meier method.

TABLE 3. Operative Methods

Operative Methods	AR-positive (No. of Patients)	AR-negative (No. of Patients)
Wedge resection	16	11
One-segmentectomy	6	1
Two-segmentectomy	2	1
Left lateral segmentectomy	4	0
Left lobectomy	0	1
Right lobectomy	2	0
Extended right lobectomy	1	0

TABLE 4. *Histopathologic Data*

Data	AR-positive (No. of Patients)	AR-negative (No. of Patients)
Tumor size		
Smaller than 2 cm	3	4
2-5 cm	17	8
5-10 cm	9	2
Larger than 10 cm	2	0
Histologic type of HCC		
Trabecular	27	11
Trabecular + clear cell	2	1
Trabecular + pseudoglandular	1	0
Trabecular + compact	1	0
Clear cell	0	1
Compact	0	1
Liver Histology		
Liver cirrhosis		
Micronodular	12	5
Macronodular	5	7
Mixed type	7	2
Chronic hepatitis	7	0

Results

AR

ARs were detected in the cytosol of HCCs taken from 31 patients. The value ranged from 2.3 to 82.6 fmol/mg protein (mean \pm SD, 19.5 ± 20.5 fmol/mg) with the dissociation constants (Kd) ranging from 4.1 to 30.9×10^{-10} M (mean \pm SD, $13.6 \pm 7.9 \times 10^{-10}$ M). The tumors removed from the remaining 14 patients possessed no detectable amount of ARs.

Operative Morbidity and Mortality

Of 31 patients with AR positive HCCs, three died after liver resection. Hepatic failure developed after relaparot-

omy for bleeding in one patient and for intra-abdominal sepsis in the other. Both patients died in hepatic coma on the eleventh and twelfth postoperative days, respectively. The third patient experienced liver failure of insidious onset and died 86 days after operation. On the other hand, two patients of the AR negative group died. The first patient died of hepatic failure on the 28th postoperative day. The second patient died of pneumonia followed by multiple organ failure on the 44th postoperative day. All the patients who died after liver resection had Child's class B liver cirrhosis. There was no difference in the morbidity and mortality rates between the two study groups.

Tumor Recurrence

In the patients with AR positive HCCs, tumor recurrence in the residual liver occurred in 19 instances 3-50 months after operation (mean \pm SD, 15.7 ± 14.4 months). On the other hand, four patients in the AR negative group had tumor recurrence 7-25 months after operation (mean \pm SD, 15.6 ± 6.5 months). Excluding the postoperative deaths, the recurrence rates were 67.9% in the former group and 33.3% in the latter group ($0.1 < p < 0.05$). Second hepatic resection was possible in eleven patients of the group with AR positive HCCs and in one patient of the AR negative group.

Long-term Survival

Cumulative survival curves for both groups of patients are shown in Figure 1. In order to discover whether the presence or absence of ARs in HCC influences the survival rate, the operative deaths were excluded. The 1- to 5-year survival rates in the AR positive and AR negative groups were 84.0% and 100%, 78.8% and 62.2%, 55.2% and 62.2%, 34.5% and 62.2%, and 17.3% and 62.2%, respectively. The difference was statistically significant during the period of 11-22 months and after 54 months after operation ($p < 0.05$).

Discussion

Although HCC usually grows very quickly and the natural history of this tumor is short, several authors reported patients who survived for a relatively long time after histologic diagnosis of HCC.^{14,15} Thus the characteristics of HCC vary from patient to patient, as in other malignant tumors.

Several investigative groups,¹⁶⁻¹⁹ including ourselves,¹⁰ have already clarified that the cytosol and nucleosol of HCC contain AR. We have shown that ARs are detectable in most of the HCCs of men and that these ARs take up extrinsically given testosterone.¹² Forbes et al.²⁰ have found objective responses in five of 25 patients with non-resectable HCCs by the treatment with an anti-androgen, cyproterone acetate. We have also obtained a similar result which will be reported elsewhere.

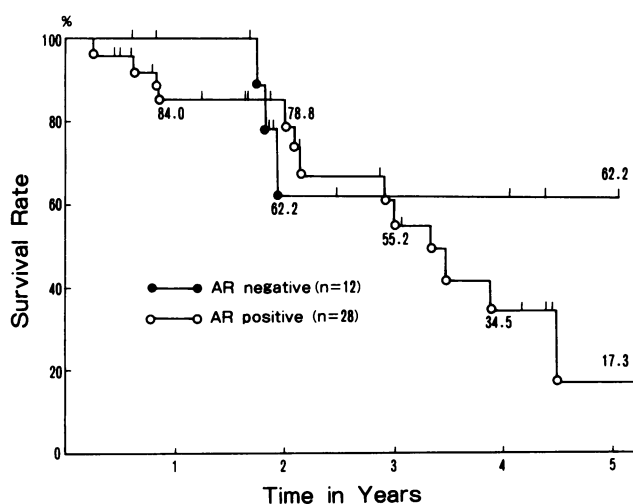


FIG. 1. Cumulative survival curves in patients with AR positive HCCs and those with AR negative tumors. The difference was statistically significant ($p < 0.05$) during the period of 11-22 months and after 54 months.

Recently, Bannister et al.²¹ and Ostrowski et al.²² reported that the development of HCC is strongly related to the increased hepatic AR concentrations in diethylnitrosamine-induced hepatocarcinogenesis of rats. Several years ago, Morris et al.²³ found that, in a hepatic carcinogenesis model in rats with 2-diacetylaminofluorene, HCC occurs more frequently in intact males and castrated females treated with testosterone than in intact females, castrated males, castrated females, and castrated males treated with diethylstilbestrol. The results of Bannister et al. and Ostrowski et al. seem to support the validity of this classical study of Morris et al. showing that HCC occurs more frequently in androgenic conditions.

As previously mentioned, several authors have suggested prognostic factors after resection of HCCs that may influence the patients' survival. Hsu et al.⁹ reported that tumor size and invasiveness were the most important factors for increasing the likelihood of survival. According to Lee et al.,⁷ better survivals were obtained in the patients who had asymptomatic tumors, noninvasive tumors, adequate surgical margin, or second or third hepatic resections. Nagao et al.⁸ observed a better survival rate in such subgroups of patients as those with a low preoperative AFP level, small tumors, or tumors with capsules. None of these authors found any difference in the survival rates of male and female patients.

Concerning the effect of sex on the long-term survival, our result was different from the above-mentioned studies. In 137 cases of radical hepatic resection, the 5-year survival rate was 52% for women but only 19% for men. This was statistically significant. We assume that this difference was reflected by the fact that AR exists in 74% of HCCs in men and in 38% of HCCs in women,²⁴ and that, throughout the postoperative period, the serum androgen level is significantly higher in men than in women.

In our present study, the prognosis of 45 patients of both sexes was evaluated in terms of the presence or absence of AR in the tumor. The recurrence rate was higher ($0.05 < p < 0.1$) and the 5-year survival rate was significantly ($p < 0.05$) lower in those with AR positive HCCs than in those with AR negative tumors. This result tallies well with the poor prognosis in male patients. In conclusion, it is likely that testosterone enhances the growth and invasiveness of HCC, which is mediated by ARs in the tumor. This characteristic will provide a potential use of anti-androgen therapy for this difficult malignant tumor.

References

- Nagasue N, Yukaya H, Hamada T, et al. The natural history of hepatocellular carcinoma: a study of 100 untreated cases. *Cancer* 1984; 54:1461-1465.
- Bengmark S, Hafstrom L, Jeppsson B, Sundqvist K. Primary carcinoma of the liver: improvement in sight? *World J Surg* 1982; 6:54-60.
- Lee NW, Wong J, Ong GB. The surgical management of primary carcinoma of the liver. *World J Surg* 1982; 6:66-75.
- Maraj R, Kew MC, Hyslop RJ. Resectability rate of hepatocellular carcinoma in rural southern Africans. *Br J Surg* 1988; 75:335-338.
- Iwatsuki S, Gordon RD, Shaw BW Jr, Starzl TE. Role of liver transplantation in cancer therapy. *Ann Surg* 1985; 202:401-407.
- Nagasue N, Yukaya H, Ogawa Y, et al. Clinical experience with 118 hepatic resections for hepatocellular carcinoma. *Surgery* 1986; 99:694-701.
- Lee C-S, Sung J-L, Hwang L-Y, et al. Surgical treatment of 109 patients with symptomatic and asymptomatic hepatocellular carcinoma. *Surgery* 1986; 99:481-490.
- Nagao T, Goto S, Kawano N, et al. Hepatic resection for hepatocellular carcinoma: clinical features and long-term prognosis. *Ann Surg* 1987; 205:33-40.
- Hsu H-C, Wu T-T, Wu M-Z, et al. Tumor invasiveness and prognosis in resected hepatocellular carcinoma: clinical and pathogenetic implications. *Cancer* 1988; 61:2095-2099.
- Nagasue N, Ito A, Yukaya H, Ogawa Y. Androgen receptors in hepatocellular carcinoma and surrounding parenchyma. *Gastroenterology* 1985; 89:643-647.
- Nagasue N, Ito A, Yukaya H, Ogawa Y. Estrogen receptors in hepatocellular carcinoma. *Cancer* 1986; 57:87-91.
- Nagasue N, Yukaya H, Chang Y-C, et al. Active uptake of testosterone by androgen receptors of hepatocellular carcinoma in humans. *Cancer* 1986; 57:2162-2167.
- Scatchard G. The attraction of proteins for small molecules and ions. *Ann NY Acad Sci* 1949; 51:660-672.
- Davidson AR, Tomlinson S, Calne RY, Williams R. The variable course of primary hepatocellular carcinoma. *Br J Surg* 1974; 61:349-352.
- Foster JH, Berman MM. The natural history of liver cancer. *In* Solid Liver Tumors. Philadelphia, London, Toronto: WB Saunders, Co., 1977; 76-79.
- Iqbal MJ, Wilkinson ML, Johnson PJ, Williams R. Sex steroid receptor proteins in foetal, adult and malignant human liver tissue. *Br J Cancer* 1983; 48:791-796.
- Wong L-Y-M, Chan S-H, Oon C-J, Rauff A. Immunocytochemical localization of testosterone in human hepatocellular carcinoma. *Histochem J* 1984; 16:687-692.
- Wilkinson ML, Iqbal MJ, Williams R. Characterisation of high affinity binding sites of androgens in primary hepatocellular carcinoma. *Clin Chim Acta* 1985; 152:105-113.
- Ohnishi S, Murakami T, Moriyama T, et al. Androgen and estrogen receptors in hepatocellular carcinoma and in the surrounding noncancerous liver tissue. *Hepatology* 1986; 6:440-443.
- Forbes A, Wilkinson ML, Iqbal MJ, et al. Response to cyproterone acetate treatment in primary hepatocellular carcinoma is related to fall in free 5 α -dihydrotestosterone. *Eur J Cancer Clin Oncol* 1987; 23:1659-1664.
- Bannister P, Parsons MA, Ingleton P, et al. Androgen receptor concentrations in the diethylnitrosamine model of hepatic carcinogenesis. *Br J Cancer* 1986; 54:857-859.
- Ostrowski JL, Ingleton PM, Underwood JCE, Parsons MA. Increased hepatic androgen receptor expression in female rats during diethylnitrosamine liver carcinogenesis: a possible correlation with liver tumor development. *Gastroenterology* 1988; 94:1193-1200.
- Morris HP, Firminger HI. Influence of sex and sex hormones on development of hepatomas and other hepatic lesions in Starin A \times C rats ingesting 2-diacetylaminofluorene. *J Natl Cancer Inst* 1956; 16:927-949.
- Nagasue N, Kohno H, Chang Y-C, et al. Androgen and estrogen receptors in hepatocellular carcinoma and surrounding liver in women. *Cancer* 1989; 63:112-116.