

## Short reports

# Cellular distribution of androgen receptors in the liver

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### Abstract

**In order to determine the cellular distribution of androgen receptors (AR) in normal liver and to examine whether phenotypic changes occur in a variety of non-neoplastic liver diseases, cryostat sections of explanted livers removed from 52 consecutive patients undergoing orthotopic transplantation were immunostained using an anti-androgen receptor monoclonal antibody. In histologically normal liver, AR was immunolocalised to the nuclei of hepatocytes. The proportion of positive hepatocytes varied from about 50% to greater than 90%. Staining, of variable intensity, was restricted to parenchymal cells with no evidence of zonal heterogeneity with respect to labelling intensity. In tissue from patients with biliary cirrhoses and in some cases of alcoholic cirrhosis, labelling for AR was observed in areas of ductular metaplasia but not in areas of "typical" ductular reaction (ductular proliferation). Otherwise, no consistent abnormalities in immunolabelling were seen in any of the diseased livers.**

(*J Clin Pathol* 1996;49:418-420)

Keywords: androgen, liver, non-neoplastic, normal, receptor.

At present, there is considerable interest in the role of androgens and their receptors in liver disease, particularly in the context of hepatocarcinogenesis. The incidence of hepatocellular carcinoma in men greatly exceeds that in women<sup>1</sup> and the hepatocarcinogenic potential of oral anabolic androgens has been documented.<sup>2</sup> In a rat model of hepatocarcinogenesis, treatment with anti-androgens reduced the size and number of tumours.<sup>3</sup> Preliminary clinical trials suggest that drugs which inhibit the activity of sex steroids may control the growth and invasiveness of hepatocellular carcinomas in selected patients.<sup>4</sup> Recently, the gender of the donor liver has been shown to influence outcome following orthotopic transplantation in adults: compared with male-male, male-female, and female-female donor-recipient gender combinations, female-male liver transplantation was associated with a significantly increased incidence of failure.<sup>5</sup>

So far, detection of the human androgen receptor has relied heavily upon ligand binding

assays. Unfortunately, these techniques are both inaccurate and insensitive, because of the lability of the receptor protein, low levels of expression and the binding of androgens to albumin and sex hormone binding globulin.<sup>6</sup> Indeed, a widely variable level of androgen receptor has been found and conflicting data from cytosolic and nuclear tumour fractions have made interpretation difficult.<sup>7</sup>

Following the cloning and sequencing of the androgen receptor gene it is now possible to produce monoclonal antibodies directed against androgen receptor with modern molecular biological techniques. Such antibodies have been used in immunohistochemical studies of prostatic<sup>8</sup> and breast carcinoma<sup>6</sup> to investigate the significance of androgen receptor status. However, similar studies addressing androgen receptor protein expression in hepatocellular carcinoma have not been reported. Indeed, very little is known of the cellular distribution of the androgen receptor protein in normal liver or of any possible alterations of this in disease states, both neoplastic and non-neoplastic.

The aims of this study were to determine the cellular distribution of androgen receptors in normal liver and to examine whether phenotypic changes occur in a variety of non-neoplastic liver diseases.

### Methods

#### LIVER SPECIMENS

The study group comprised explanted livers removed from 52 consecutive patients undergoing orthotopic transplantation at the Freeman Hospital, Newcastle-upon-Tyne, UK. Wedge samples of liver explants were snap-frozen within 20 minutes of removal from the body in OCT tissue embedding medium. Specimens were stored air-tight at  $-70^{\circ}\text{C}$  until used for immunohistochemistry. Histopathological assessment was carried out using routine formalin fixed, paraffin wax embedded sections. Patient details are shown in table 1. Histologically normal liver was available in three cases. Transplantation was carried out in two patients for metastatic carcinoma and areas of non-tumorous liver were sampled. In a further case, complications following surgery for non-hepatic pathology necessitated transplantation.

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Accepted for publication  
12 December 1995

Table 1 Immunohistochemical detection of androgen receptors in explanted livers with a monoclonal antibody directed against the androgen receptor

Histological diagnosis	n	Mean (SD) age in years	Men		Women	
			AR positive	AR negative	AR positive	AR negative
Primary biliary cirrhosis	21	55.7 (8.23)	1	2	13	5
Alcoholic cirrhosis	12	48.4 (8.15)	1	6	4	1
Primary sclerosing cholangitis	5	41.4 (12.4)	2	2	1	—
Autoimmune chronic hepatitis	4	40.8 (2.22)	1	—	1	2
Hepatic artery thrombosis	2	36.5 (3.54)	—	1	—	1
Paracetamol overdose	1	23	—	1	—	—
Cryptogenic cirrhosis	1	66	—	1	—	—
Cholangiocarcinoma	1	49	—	1	—	—
Congenital hepatic fibrosis	1	40	—	—	1	—
Fulminant hepatic failure	1	38	1	—	—	—
Normal	3	38.3 (9.50)	1	—	2	—

AR = androgen receptor.

#### IMMUNOHISTOCHEMICAL DETECTION OF ANDROGEN RECEPTORS

Immunohistochemical detection of androgen receptors was performed on cryostat sections using a commercially available mouse monoclonal antibody directed against androgen receptor (clone F39.4.1; Novocastra Laboratories, Newcastle, UK) with a standard peroxidase-antiperoxidase complex method (PAP). This antibody was raised against a synthetic peptide corresponding to amino acids 301–320 of the N-terminal domain of human androgen receptor.<sup>9</sup> It has been characterised in western blot analysis and its specificity for the androgen receptor, as distinct from other steroid hormone receptors, established.

Briefly, 6 µm cryostat sections, mounted on 3-aminopropyltriethoxysilane coated slides, were immediately fixed with Zamboni's fluid for 10 minutes. Following washing with Tris buffered saline (TBS) (3 × 10 minutes), sections were incubated with normal rabbit serum diluted 1 in 10 in TBS (pH 7.4) for 15 minutes at room temperature to minimise non-specific binding of reagents in subsequent steps. Incubation with the monoclonal antibody directed against androgen receptor, diluted 1 in 5 in TBS containing 0.5% bovine serum albumin and 0.1% sodium azide (pH 7.8), was carried out overnight at 4°C. After washing in TBS (2 × 10 minutes), sections were incubated with 0.5% hydrogen peroxide/methanol for 10 minutes at room temperature to block any endogenous peroxidase activity. Subsequent washing with TBS for 10 minutes was followed by incubation with unconjugated rabbit anti-mouse IgG, diluted 1 in 20 in TBS, for 30 minutes at room temperature. Further washing in TBS (2 × 10 minutes) was followed with incubation for 30 minutes at room temperature with mouse PAP complexes diluted 1 in 50 in TBS. After washing the sections in TBS (2 × 10 minutes), peroxidase activity was developed using 3,3'-diaminobenzidine containing 0.03% hydrogen peroxidase for three minutes at room temperature. Sections were counterstained with haematoxylin. Sections of prostatic tissue were used as a positive control.

#### Results

In the three cases of histologically normal liver androgen receptors were immunolocalised to hepatocytes, where labelling was observed within nuclei. Staining, of variable intensity and

less than that observed in the control prostatic tissue, was restricted to parenchymal cells with no evidence of zonal heterogeneity with respect to labelling intensities. No immunolabelling was observed in sinusoidal cells, bile duct epithelial cells or vascular endothelium. The proportion of positive hepatocytes in each of the three explants varied from about 50% to greater than 90%.

Androgen receptors were demonstrated in 26 (53%) of 49 explanted livers with non-neoplastic pathology (table 1). In tissue from patients with biliary cirrhoses (primary biliary cirrhosis and primary sclerosing cholangitis) and in some cases of alcoholic cirrhosis, labelling for androgen receptors was observed in areas of ductular metaplasia but not in areas of "typical" ductular reaction (ductular proliferation). Otherwise, no consistent abnormalities in immunolabelling were seen in any of the diseased livers. No staining was observed in bile ducts infiltrated by inflammatory cells and no relation was seen—for example, in areas of piecemeal necrosis, between the hepatocyte staining intensity and the degree of inflammation. In those cases with demonstrable androgen receptors, the proportion of positive hepatocytes in any one case ranged from about 20% to greater than 90%. The mean age of androgen receptor positive and negative cases did not differ significantly (49.3 v 49.0 years, respectively). The proportion of female cases, as a whole, positive for androgen receptor was significantly greater than that for males (69.0 v 30.0%,  $p < 0.02$   $\chi^2$  test with Yates' correction). Within individual diagnostic groups, however, the small numbers of patients precluded further statistical analysis.

#### Discussion

This is the first study to assess systematically the cellular distribution of androgen receptors in normal and diseased liver. Using ligand binding assays several authors have attempted to determine whether androgen receptor status is of prognostic or therapeutic importance in hepatocellular carcinomas and to assess how the expression of androgen receptor might alter during the progression from normality to malignancy.<sup>7</sup> However, such studies have been inconclusive, reflecting both methodological problems and limited material. In this study expression of androgen receptors was found to

be restricted to hepatocytes in both normal and diseased liver. By immunohistochemistry, no consistent abnormalities in androgen receptor expression were observed in non-neoplastic chronic liver diseases. Interestingly, androgen receptor expression was observed in a significantly greater proportion of women.

Recently Negro *et al*<sup>10</sup> developed a non-radioisotopic in situ hybridisation assay specific for the human androgen receptor mRNA. Although no normal liver was examined, androgen receptor mRNA was detected in eight (42%) of 19 non-neoplastic liver specimens, a similar proportion to that found in this study. Androgen receptor mRNA was similarly restricted to hepatocytes, although the proportion of reactive hepatocytes found was noticeably less than in the present study and was never more than 10%. This difference most probably reflects a difference in sensitivity of the two techniques. Indeed, in a test set of frozen sections from five hepatocellular carcinomas (HCCs) stained with a different androgen receptor monoclonal antibody, Negro *et al* were able to demonstrate androgen receptor protein in only one case, which also expressed androgen receptor mRNA. Two HCCs, strongly positive for the androgen receptor mRNA, were negative by immunohistochemistry and in the remaining two cases, neither androgen receptor protein nor its mRNA could be detected.

Several clinical and experimental observations suggest that drugs which inhibit the activity of sex steroids may control the growth and invasiveness of HCCs in selected patients. However, results of pilot clinical trials have been disappointing so far.<sup>3,4,10</sup> One explanation for this may be the lack of accurate evaluation

of hormonal status prior to any such treatment. The present study has shown the potential of immunohistochemical techniques to demonstrate androgen receptor in liver tissue. We believe that further studies of androgen receptor expression in premalignant lesions, such as small cell dysplasia and atypical macro-regenerative nodules, and in hepatocellular carcinoma are now warranted.

We gratefully acknowledge the help of the physicians and surgeons of the Liver Transplant Group, Freeman Hospital Trust, for collection of material used in the study. SAH was in receipt of a Medical Research Council Clinical Training Fellowship.

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*J Clin Pathol* 1996;49:420-422

## Alpha-fetoprotein production by a hepatoid adenocarcinoma of the uterus

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### Abstract

**A case of a 62 year old Japanese woman with an endometrial adenocarcinoma producing alpha-fetoprotein (AFP) is described. Microscopically, the tumour was composed of a major medullary portion and a minor tubular adenocarcinoma which had invaded the myometrium, the myometrial lymphatics and blood vessels. Neoplastic cells in the medullary portion were polygonal with glycogen-rich cytoplasm. Vascular permeation by neoplastic cells was prominent. Extensive hepatoma-like features were observed. The tumour cells lacked features suggestive of a diag-**

**nosis of embryonal carcinoma or endodermal sinus tumour. The production of AFP by the tumour cells was demonstrated immunohistochemically using the PAP technique. Only two cases of AFP producing endometrial adenocarcinomas have been reported previously.**

*(J Clin Pathol* 1996;49:420-422)

**Keywords:** alpha-fetoprotein, endometrial adenocarcinoma, hepatoid adenocarcinoma.

Alpha-fetoprotein (AFP) is a fetal serum protein synthesised by fetal liver, yolk sac and gastrointestinal tract. After birth, AFP dis-

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Accepted for publication  
18 October 1995