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GROWTH FACTORS, ENDOCRINE ASPECTS AND HORMONAL TREATMENT IN HEPATOCELLULAR CARCINOMA—AN OVERVIEW

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Summary—Several clinical observations suggest that hepatocellular carcinoma (HCC or "hepatoma") may be a hormone-dependent tumour; the apparent relation to anabolic steroids and oral contraceptive preparations, and the striking male predominance particularly among patients with cirrhosis. In many animal models thyroid hormones, prolactin and testosterone stimulate tumour growth, and the latter may enhance the progression of chemically-induced hyperplastic nodules to frank malignancy. In animals and humans, both oestrogen and androgen receptors have been reported in normal and malignant liver tissue though some of the evidence is conflicting and the amounts detected vary widely. From a therapeutic standpoint, we failed to show any advantage from the addition of tamoxifen to adriamycin, in a controlled trial although other workers have, more recently, reported prolonged survival using tamoxifen alone. About 20% of HCC patients receiving the antiandrogen cyproterone acetate showed a clinical response.

Worldwide, hepatocellular carcinoma (HCC) remains one of the major problems in clinical oncology. It is probably the commonest tumour in men and, being highly malignant, usually leads to death within one year of the onset of symptoms. Young people are often affected and, in most cases, there is no effective treatment [1]. Any prospects for control by hormonal manipulation would therefore be extremely welcome.

About 80% of patients with HCC have coexistent chronic liver disease, usually at the stage of cirrhosis, and while the aetiological implications of this observation remain controversial, the importance in relation to the hormonal environment is clearer. For example, sex hormone binding globulin (SHBG) concentrations are raised in patients with cirrhosis, affecting "free" levels of the sex hormones. In the early stages of cirrhosis, testosterone levels are within the reference range or even higher but become progressively lower as the disease decompensates. On the other hand the oestradiol concentration is initially within the reference range and may become increased [2]. Thus abnormalities of sex hormone concentrations in patients with HCC may be caused by the coexistent cirrhosis. Additionally the hormonal environment at the start of tumour development may be very different to that when it becomes clinically manifest.

With this background, it is our aim to review briefly: the data implicating endocrine factors in the development and growth of human and experimental hepatocellular carcinoma; steroid and peptide hormone receptor-ligand interactions and the rapidly expanding field of growth factors; the effects of malignant change on hormone binding proteins and finally hormonal treatment in man.

IS HEPATOCELLULAR CARCINOMA A SEX HORMONE-DEPENDENT TUMOUR?

It has been long established that in certain mouse strains, administration of testosterone significantly increases the frequency with which chemically-induced hyperplastic nodules progress to hepatoma. Similarly castration of male animals decrease the frequency of this occurrence [3]. In man, however, the situation is less clear.

A role for sex hormones in the development and the progression of hepatocellular carcinoma is based upon the association of HCC with

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patient usage of oral contraceptive preparations and anabolic steroids as well as male predominance. However, too much weight is placed on these observations. The association with the oral contraceptive preparations and anabolic steroids is both rare and tenuous. Similarly, whilst it is undoubtedly true that in Western populations there is a pronounced male predominance, this is confined to those with cirrhosis—the male:female ratio being similar in non-cirrhotic patients [4].

We have followed up prospectively nearly 1000 patients with different types of cirrhosis for up to 10 yr and multivariate analysis showed that one of the major independent risk factors for the development of HCC was male sex [5]. In a recent report from Taiwan, it was shown that the predominance amongst males with associated cirrhosis is also evident in young children [6]. This might suggest that it is not simply circulating hormone levels that are important in encouraging tumour growth in males. Other possibilities include sexual dimorphism with respect to metabolism of environmental carcinogens. For example, the pathway leading to the active metabolite of aflatoxin is much more active in certain male animals in comparison to females [7].

RECEPTOR–LIGAND INTERACTIONS IN LIVER NEOPLASIA

Steroid hormones

Oestrogen receptors (ER) have been identified in both cytoplasmic and nuclear preparations of liver tissue. ER can be located in fetal, normal adult and cirrhotic liver with even higher levels of receptor being recorded in HCC tissue [8–10]. Thus binding of ER to specific receptors located within pathological liver tissue and activation of receptor–ligand pathways might be expected to influence tumour progression.

Cytosolic and nuclear androgen receptors (AR) have also been recorded in both HCC tissue and fetal liver but appear to be absent in adult cirrhotic liver [9, 10]. The presence of AR in fetal liver suggests that androgens have a role to play in fetal liver development and that the re-expression of these receptors at the adult stage could either initiate or contribute to the tumour process. Moreover, AR have been identified in the androgen-dependent Morris Hepatoma liver tumour [11]. However the androgen dependency of the normal liver is still disputed by many investigators because of conflicting reports on the presence of AR [9, 12, 13]. Much of the controversy arises as a result of difficulties in AR measurement in human tissue, a process which is often hampered by lack of sufficient material for use in standard assays such as Scatchard analysis. Proposed use of a single saturating dose analysis on liver needle biopsies may well provide a more consistent and systematic testing system for the presence of cytoplasmic AR in HCC and other human liver tumours as well as normal liver [14].

There is also some evidence of a role for glucocorticoids in HCC. The absence of glucocorticoid receptor in most cases of cirrhotic liver [15], contrasts the presence of the receptor in both normal fetal liver tissue and HCC [15, 16]. The role of progesterone in tumour progression is, however, less clear. At high doses (500 μg/day) the steroid has been shown to stimulate hepatoma (Morris) growth in male rats bearing MH 7787, although it failed to do so at lower doses. However, since cytoplasmic progesterone receptors have been found absent in tumour tissue this effect was felt to be due to the residual androgenic activity of the progestational compounds used in the experiments [11]. Thus although evidence suggests that HCC is a steroid-responsive tumour, further analysis of both cytoplasmic and nuclear receptor content within tumour tissue is necessary for a comprehensive understanding of the role of sex steroid hormones in the development and progression of HCC.

Peptide hormones

Induction of hypothyroidism significantly inhibits the growth of established experimental hepatoma and results in the increased survival of the test animals [17–20]. Additionally both thyroxine and prolactin stimulate Morris hepatoma growth [20]. Growth hormone (GH), on the other hand, has been shown to be necessary for the optimal growth of a rat hepatoma cell line [21] while failing to stimulate growth of Morris hepatoma tissue [20]. These investigations could be explained in the light of recent ones on the content of GH receptors [22] within human HCC tissue and surrounding cirrhotic lobules. Results demonstrated a lack of GH receptors in tumour tissue in contrast to the presence of both cytosolic thyroid hormone and prolactin receptors, pointing to a regulatory role for prolactin and thyroid hormone but probably not GH in liver neoplasia.

GROWTH FACTORS

The autocrine mechanism of cellular transformation is thought to be the most frequent initiator of tumorigenesis. Here cells that possess specific receptors for the polypeptides they secrete, produce either or both ligand and receptor in an uncontrolled fashion. IGF-II transcripts, synthesised at high levels in human and rodent fetal liver, but not in the adult [23–29], are over-expressed in certain embryonic tumours [24, 25] and other liver neoplasia [24, 25, 30–33]. Increased expression of IGF-II mRNA in both Hep G2 cells (derived from a human hepatoblastoma) [24, 25, 30, 31] and human HCC [34] also accompanies a differential re-expression of fetal transcripts representing a return of liver cells to a dedifferentiated state such as that found during neoplastic transformation.

Hepadna viruses have for some time been thought to play a major role in liver cancer in humans and animals, and Gu [35] postulated a non-stochastic mechanism for the role of HBV in the regulation of c-myc, ets-2 and IGF-II in hepatic proliferation, by suggesting that expression of these factors could be triggered by HBV or chemical carcinogens. Increased IGF-II mRNA transcripts have been reported in HCC developing in woodchucks infected with hepadna virus [32] and a recent report in abstract form [36] has documented upregulation of IGF-II gene expression at the transcriptional level in tumorous tissue while HBV transcripts were recorded in the peritumorous areas. Recently our own data using *in situ* hybridization has shown that the expression of IGF-II mRNA is upregulated in HCC tissue where there is cirrhosis and when the patients are carriers of the hepatitis B virus (C. N. d'Arville, unpublished observations). Thus increased IGF-II mRNA transcripts could represent a premalignant proliferative state, such as that found in virus-induced cirrhosis and certain benign liver tumours. Two mechanisms; one involving ligand interaction with specific receptors present within the cells, the other reduction of insulin-like growth factor binding proteins {(IGFPB) [37], which bind IGFs and control the level of these molecules presented to the liver cells} could lead to stimulation or inhibition of growth and metabolism of the already activated cells and promote tumour development.

Paracrine mechanisms of cellular transformation have also been cited in the liver. Certain studies in human fetal liver using *in situ* hybridization, have suggested growth factor synthesis occurs mainly in non-parenchymal, perisinusoidal liver cells [38]. Aberrant IGF-I receptor expression has been demonstrated in amongst others; hepatocytes from human hepatoma, fetal human liver and regenerating rat liver [38]. Thus since HCC generally develops in a liver with pre-existing cirrhosis, regenerating nodules separated by fibrous tissue, could undergo malignant transformation as a consequence of growth factors such as IGF-I and -II, produced by perisinusoidal cells, feeding back to receptors located within the regenerating parenchyma. Platelet-derived growth factor (PDGF) via its receptor, found present in certain hepatoma cell lines [40], has also been implicated in paracrine mechanisms of cellular transformation, while another growth factor of potential importance to the liver is transforming growth factor beta (TGF-β). This growth factor is the product of an oncosuppressor gene and suppresses certain cancer phenotypes [41]. It has been reported that levels of TGF-β-mRNA remain unchanged in benign, cirrhotic and malignant human liver [34] but regulation of receptor levels for TGF in liver disease is still unknown.

Reduced epidermal growth factor receptor [EGFr] expression has been demonstrated in rat preneoplastic and neoplastic [42–45] liver conditions. Furthermore our own studies have recently demonstrated that gene expression for EGFr in hepatocytes from the cirrhotic rat liver, at both transcriptional (C. N. d'Arville, unpublished observations) and translational [46] levels, is also reduced. These results contradict an autocrine role for the growth factors TGF and EGF via EGFr in neoplastic transformation. However, EGFr, found on some fibroblasts [47] and which may be present in non-parenchymal liver cells, could be implicated in paracrine loops. The fact that c-erb-B2 (an oncogene that upon expression produces a protein sharing homology with the EGFr) expression is highest during certain stages of fetal liver development suggests that this oncogene could be involved in the control of human liver cellular proliferation and neoplasia. Moreover, oncogene amplification and alteration has been reported in both human hepatoma and liver cirrhosis [48]. However, levels of c-erb-B2 have been reported as unaltered in HCC compared to normal tissue by others [35] and therefore the role of this and other oncogenes, in liver neoplasia has yet to be clarified.

ABNORMALITIES OF HORMONE BINDING PROTEINS

The increased concentration of SHBG and other hormone binding globulins such as thyroxine binding globulin (TBG) has been noted in patients with chronic liver disease, while even more pronounced abnormalities can be seen in patients with HCC. For example, the serum concentration of thyroxine (T_4) or triiodothyronine (T_3) is grossly raised in about 40% of patients with HCC and this is clearly accountable for by the increased levels of TBG [49, 50]. Rising concentrations of T_4 and TBG can be detected in cirrhotic patients several years before tumour development becomes clinically manifest. In contrast, those who die without HCC have falling TBG levels. Thus increased production of TBG might be one of the earliest serological events during the development of HCC. In addition, the TBG produced by malignant hepatocytes also has a markedly reduced ability to bind thyroid hormones (as measured by the binding ratio). This abnormality can also be detected long before tumour becomes clinically apparent [51].

The precise underlying mechanism for this has not yet been elucidated, but appears to be part of a generalised abnormality in the serum glycoprotein structure in patients with HCC [52]. Alpha fetoprotein, which is a hormone binding protein in some animals, exhibits increased fucosylation when it is derived from malignant hepatocytes, and this appears attributable to an imbalance between fucosidase and fucosyltransferase enzymes [53].

HORMONAL TREATMENT IN MAN

In 1982 Friedman *et al.* reported remissions in 2 out of 5 patients with HCC treated with progestins (megesterol or methoxyprogesterone [54]). The same group also found that the combination of doxorubicin (the only cytotoxic agent to demonstrate a consistent, albeit small, response rate in HCC) and tamoxifen extended the median survival of 12 patients to greater than 8 months [55]. In 1987 we reported a prospective randomised controlled trial involving 59 patients and comparing tamoxifen plus doxorubicin with doxorubicin alone. The first patient to enter the study responded dramatically and died 3 yr later of an unrelated illness. However, over the next 2 yr we saw no further dramatic responses and indeed, in the final analysis, there was no difference in the response rate and the survival experience of the two groups [56].

A much more optimistic result using tamoxifen alone was obtained by Farinati *et al.* [57] and reported in abstract form in 1989. These workers reported that survival at 1 yr was 40% in the tamoxifen (30 mg/day) treated group compared to zero in the untreated control group ($P < 0.001$), although there was no suggestion of any response in the acknowledged sense but rather a slowing of tumour growth. If these results are confirmed they represent an exciting advance.

We have also tried the alternative approach of using the antiandrogen cyproterone acetate (up to 300 mg/day) in 25 cases. In all 5 patients with objective evidence of a response there was an associated fall in free 5α-dihydrotestosterone [58].

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