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Hepatocellular Carcinoma, Steroid Hormones and Metalloproteases

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1. Introduction

Liver cancer is the fifth and seventh most frequently diagnosed cancer worldwide in men and women, respectively, but the second most frequent cause of cancer death in men [1]. In addition, hepatocellular carcinoma (HCC) represents the major histological subtype of primary liver cancers, accounting up to 90% of the total liver cancer burden worldwide [2]. The incidence of HCC has increased significantly over the past 10 years and is expected to increase because of the actual high prevalence of viral hepatitis C-seropositive individuals and also because of the known long latency period to HCC development from the initial hepatitis C virus (HCV) infection, which may take 2-3 decades [3]. Despite of the varied treatment options, the prognosis of HCC remains poor. Thus, estimated 5-year survival rates are in the range of 26% to 50%, and disease-free survival is 13% to 29% [4]. At present, systemic chemotherapy is quite ineffective in HCC treatment, and is also known to express the multidrug-resistance gene MDR-1 [5]. Therefore, is necessary to identify and characterize molecular abnormalities of clinical significance in HCC. Besides the heterogeneity of different HCC subtypes, these tumors may use different cellular pathways and oncogenic mechanisms at different development stages, and this is of essential importance to develop biologically-based clinical trials.

2. Steroid hormone receptors and hepatocellular carcinoma

Although sex differences in liver cancer may be attributed to differences in lifestyle [6-8], there are several epidemiological and experimental studies suggesting that HCC might be, in part, hormone-related. Liver cancer is predominantly a male disease, with approximately three

times higher incidence and mortality among men than women [9]. This male predominance is further supported by the fact that chronic liver disease progresses more rapidly to cirrhosis in males than in females, and therefore cirrhosis-derived HCC is largely seen in men and postmenopausal women disease [10].

The role of estrogens in modulating morphological and physiological features of liver became evident in early 1970s when a possible correlation between occurrence of hepatic neoplasms and use of oral contraceptives was suggested [11-12]. On the basis of these data, several *in vitro* and *in vivo* studies have explored the importance of sex hormones in HCC. Animal model-based studies indicate that sex hormones play a key role in tumor progression, showing that ovarian estrogens protect against tumor progression, whereas androgens promote tumorigenesis [13-15]. It has been reported that the protective effect of estrogens against chemically induced liver tumors is mediated by prolactin (PRL) through liver prolactin receptors (PRLR) [16-18]. Nevertheless, the precise role of male and female sex hormones and their receptors in HCC remains still poorly understood. There is not enough information regarding the mechanism of estrogen and androgen in HCC.

Estrogen and androgen mediate their biological functions through binding to their specific receptors, the estrogen receptor (ER) and the androgen receptor (AR). Both ER and AR belong to the nuclear receptors family that, as transcription factors, regulate the expression level of several genes such as those involved in triggering immune responses, cell proliferation and apoptosis [19-21]. Therefore, sex hormones play a key role in normal physiology of organs other than these of the reproductive system.

Variable expressions of ER and AR has been found in normal liver and HCC using different methods, such as immunohistochemistry, enzyme-immuno assays, or determining mRNA levels (Table 1 and Table 2 give the details of these studies for ER and AR, respectively), which indicate a relationship between sex hormones and pathogenesis of HCC. The percentage of positivity for these receptors varied between the different studies [22]. These differences may be due to several methodological aspects, such as differences in the origin of the studied patients populations, sample types or technologies determining the receptor expression. In a recent study, we determined the expression of sex hormones receptors in 31 HCC patients by immunohistochemistry using tissue micro-arrays technology [23]. Our results demonstrate a wide variability in the immunohistochemical values for steroid receptors among HCCs: 67.7% of tumors stained positively for AR, 51.6% for ER and 83.8% for progesterone receptor (PgR), but, among the positive cases, immunostaining score values for each protein were largely variable.

Reference	Protein /mRNA	Type of liver tissue	Method	Localization	n	% positive cases	Country	Year
[122]	ER protein	Normal	BA	cytosolic	4	100	United Kingdom	1978
[123]	ER protein	Normal	BA	-	3	100	Germany	1978
[124]	ER protein	Normal	BA	-	2	100	United States	1982

Reference	Protein /mRNA	Type of liver tissue	Method	Localization	n	% positive cases	Country	Year
[125]	ER protein	Normal	BA	-	6	100	Germany	1982
[126]	ER protein	Normal	BA	-	6	100	United States	1983
[127]	ER protein	Normal	BA	cytosolic and nuclear	4	100	United Kingdom	1983
[128]	ER protein	Surrounding	BA	cytosolic	30	43	Japan	1986
[129]	ER protein	Non-cancerous	BA	cytosolic and nuclear	7	43	Japan	1986
[130]	ER protein	Non-cirrhotic	EIA	cytosolic	12	12	Japan	1987
[131]	ER protein	Normal		-	2	2	Japan	1988
[132]	ER protein	Surrounding	BA	cytosolic	17	65	Japan	1989
[133]	ER protein	Surrounding			22	64	Japan	1989
[134]	ER protein	Surrounding	BA	cytosolic	5	100	Japan	1990
[135]	ER protein	Normal	BA		9	9	United States, Italy	1991
[52]	ER protein	Non-cancerous	BA	cytosolic	26	42	Spain	1993
[136]	ER mRNA	Non-cancerous	ISH		13	54	Italy	1993
[137]	ER mRNA	Peritumoral	RT-PCR	-	32	88	Korea	2006
[124]	ER protein	HCC	BA	cytosolic	5	100	United States	1982
[127]	ER protein	HCC	BA	cytosolic and nuclear	5	100	United Kingdom	1983
[138]	ER protein	HCC	IHC	cytosolic	10	10	Singapore	1984
[128]	ER protein	HCC	BA	cytosolic	30	40	Japan	1986
[139]	ER protein	HCC	BA	cytosolic and nuclear	8	13	Japan	1986
[130]	ER protein	HCC	EIA	cytosolic	13	38	Japan	1987
[132]	ER protein	HCC	BA	cytosolic	19	37	Japan	1989
[24]	ER protein	HCC	BA	cytosolic	66	39	Japan	1990
[134]	ER protein	HCC	BA	cytosolic	6	17	Japan	1990
[140]	ER protein	HCC	BA	cytosolic	21	48	Japan	1991
[141]	ER protein	HCC	BA	cytosolic and nuclear	9	89	United States, Italy	1991
[135]	ER protein	HCC	BA	cytosolic and nuclear	9	89	United States, Italy	1991

Reference	Protein /mRNA	Type of liver tissue	Method	Localization	n	% positive cases	Country	Year
[52]	ER protein	HCC	EIA	cytosolic	26	15	Spain	1993
[136]	ER protein	HCC	IHC	-	15	0	Italy	1993
[136]	ER mRNA	HCC	ISH	-	15	73	Italy	1993
[142]	ER mRNA	HCC	RT-PCR	-	14	57	Italy	1995
[25]	ER protein	HCC	EIA	cytosolic	33	39	Germany	1997
[143]	ER protein	HCC	IHC	cytosolic	71	24	China	1997
[144]	ER mRNA	HCC	RT-PCR	-	40	70	Italy	1998
[145]	ER mRNA	HCC	RT-PCR	-	42	60	Italy	2003
[146]	ER protein	HCC	IHC	cytosolic and nuclear	45	71	United States/ Koreans	2004
[147]	ER protein	HCC	IHC	-	28	39	China	2004
[147]	ER mRNA	HCC	RT-PCR	-	28	89	China	2004
[137]	ER mRNA	HCC	RT-PCR	-	32	100	Korea	2006
[148]	ER protein	HCC	IHC	-	66	5	Mexico	2007
[23]	ER protein	HCC	IHC	Nuclear	31	52	Spain	2007

ER: Estrogen receptor; HCC: Hepatocellular carcinoma; BA: Binding assay; EIA: Enzyme immunoassay; ISH: *In situ* hybridization; RT-PCR: Reverse transcriptase-polymerase Chain reaction for ER α wild type; IHC: Immunohistochemistry.

Table 1. Estrogen expression in normal liver and hepatocellular carcinoma tissue samples.

Reference	Protein /mRNA	Type of liver tissue	Method	Localization	n	% positive cases	Country	Year
[127]	AR protein	Normal	BA	cytosolic and nuclear	4	0	United Kingdom	1983
[139]	AR protein	Non-cancerous	BA	cytosolic and nuclear	6	17	Japan	1986
[132]	AR protein	Non-cancerous	BA	cytosolic	17	65	Japan	1989
[133]	AR protein	Surrounding			21	33	Japan	1989
[134]	AR protein	Surrounding	BA	cytosolic	10	80	Japan	1990
[135]	AR protein	Normal	-	-	9	100	United States, Italy	1991
[137]	AR mRNA	Peritumoral	RT-PCR		32	100	Korea	2006

Reference	Protein /mRNA	Type of liver tissue	Method	Localization	n	% positive cases	Country	Year
[127]	AR protein	HCC	BA	cytosolic and nuclear	5	100	United Kingdom	1983
[138]	AR protein	HCC	IHC	cytosolic	10	50	Singapore	1984
[149]	AR protein	HCC	BA	cytosolic	19	74	Japan	1985
[150]	AR protein	HCC	BA	cytosolic and nuclear	5	100	United Kingdom	1985
[139]	AR protein	HCC	BA	cytosolic and nuclear	8	50	Japan	1986
[151]	AR protein	HCC	BA	cytosolic	13	100	United Kingdom	1988
[132]	AR protein	HCC	BA	cytosolic	19	37	Japan	1989
[27]	AR protein	HCC	BA	cytosolic	45	69	Japan	1989
[134]	AR protein	HCC	BA	cytosolic	11	64	Japan	1990
[140]	AR protein	HCC	BA	cytosolic	21	86	Japan	1991
[135]	AR protein	HCC	BA	cytosolic and nuclear	9	100	United States, Italy	1991
[152]	AR protein	HCC	BA	cytosolic	5	100	Japan	1992
[52]	AR protein	HCC	BA	cytosolic	26	54	Spain	1993
[28]	AR protein	HCC	BA	cytosolic	43	65	Spain	1995
[23]	AR protein	HCC	IHC	nuclear	31	68	Spain	2007
[129]	AR mRNA	HCC	ISH	-	22	73	Italy	1994
[153]	AR mRNA	HCC	RT-PCR	-	38	89	Italy	2002
[137]	AR mRNA	HCC	RT-PCR	-	32	100	Korea	2006

AR: Androgen receptor; HCC: Hepatocellular carcinoma; BA: Binding assay; ISH: *In situ* hybridization; RT-PCR: Reverse transcriptase-polymerase Chain reaction; IHC: Immunohistochemistry.

Table 2. Androgen receptor expression in normal liver and hepatocellular carcinoma tissue samples.

Several studies have analyzed the relationship between ER or AR and clinicopathological parameters from HCCs patients and their clinical outcome (Table 3). In general, ER expression was associated with higher tumor aggressiveness and/or worse prognosis in HCC patients [24-26], whereas AR expression was negatively associated with recurrence [27-29]. It is remarkable the finding that the presence of the ER α variant has been associated with shortened overall survival in patients with resectable HCC [30,26].

3. Steroid receptors and hepatitis virus

It is of special interest the interaction between sex estrogen receptors and viral proteins in hepatitis B virus (HBV) and hepatitis C virus (HCV)-induced HCC. It has been reported that a HBV protein (HBx) interacts with ER α . HBx is a multifunctional protein involved in neoplastic transformation in cultured cells and in HCC induction in transgenic mice. Both HBx and vER α (Delta 5 deletion variant of ER α) have additive effects on suppressing ER α transactivation [31]. In addition, it was reported that tamoxifen inhibits ER α actions and suppresses HCV genome replication [32], which may be of potential interest to develop new anti-HCV strategies based on anti-ER drugs. On the other hand, higher serum androgen concentrations or a specific AR gene, which leads to higher AR activities, have also been associated to higher risk in HBV-mediated HCC [33-34]. Likewise, it has been reported that the combination of male gender and HBV infection had a significant synergistic effect on HCC progression [35]. Most recently, Ming-Heng *et al.* found that hepatic AR increases the HBV viral titer by enhancing HBV RNA transcription through direct binding to the androgen response element near the viral core promoter. This activity forms a positive feedback mechanism with the cooperation of its downstream target, the HBx protein, to promote hepatocarcinogenesis. In addition in these same study administration of a chemical compound that selectively degrades AR, ASC-J9, was able to suppress HCC tumor size in a transgenic HBV mouse model that developed HCC upon exposure to a low dose of N'-N'-diethylnitrosamine (DEN). These results demonstrate that targeting the AR, rather than the androgen, could be developed as a new therapy to battle HBV-induced HCC [36].

Reference	Protein / mRNA	clinicopathological parameters	Correlation	n	year	country
[128]	ER protein	Serum alpha-fetoprotein, HBV profile, tumor histology, carcinoembryonic antigen	-	30	1986	Japan
[132]	ER and AR protein	Serum alpha-fetoprotein, Histopathology, HBV markers	-	19	1989	Japan
[24]	ER protein	Sex, age, alcohol consumption, hepatic function, other liver disease, tumor size, hepatic resection, tumor recurrence, long-term survival rate	ER- patients showed higher rate of resection and larger tumor size	66	1990	Japan
[49]	ER and AR protein	Local recurrence	AR expression was associated with intrahepatic recurrence	78	1995	Japan
[25]	ER protein	Survival after curative resection	ER+ tumor have a negative effect on patient survival after curative resection	28	1997	Germany

Reference	Protein / mRNA	clinicopathological parameters	Correlation	n	year	country
[26]	ER mRNA	Survival	Wild type ERs tumors showed long survival in patients	96	2000	Italy
[132]	AR protein	Recurrence and survival rate	AR+ patients showed higher recurrence rates. AR- patients showed better survival rates.	45	1989	Japan
[28]	AR protein	Tumor size and tumor recurrence	AR expression correlated with smaller tumor size. Higher tumor recurrence in AR+ surrounding tissues.	43	1995	Spain
[29]	AR protein	Tumor size and survival time	AR+ tumor correlated with higher tumor size and lower survival rates.	32	1998	China

ER: Estrogen receptor; AR: Androgen receptor

Table 3. Relationship between estrogen or androgen receptors with clinicopathological parameters and clinical outcome of patients with HCC.

4. Endocrine therapy in hepatocellular carcinoma

Considering both the epidemiologic and the experimental data supporting the sex steroids influence in growth and progression from HCCs, different clinical studies analyzed if endocrine therapy could be interesting in advanced disease. Table 4 gives details of some of these studies. Trials using several agents, such as the anti-estrogen tamoxifen, megestrol acetate, progestin, the gonadotropin releasing hormone (GnRH) agonist leuporelin and the anti-androgen flutamide have been uniformly disappointing [37-40]. At present, experts have concluded that hormonal manipulation should not be a part of the current management of patients with HCC [41-42]. However, it is possible that the rational design of an endocrine therapy requires a more complete understanding of the role of sex hormones in the tumorigenic process and how hormones and organ systems interact during this process [18], in addition to careful selection of the new studies patient populations, since patient stratification based on gender may uncover signals of activity of hormonal therapy in these settings. Notably, it has been shown that the anti-estrogens tamoxifen and raloxifene, behave differently in different tissues, producing estrogen-agonist activity in one tissue while behaving as an antagonist in another [43-44]. In addition, *in vitro* studies in the liver cell line Hep3B, indicate that raloxifene induces the insulin-like growth factor (IGF-I) gene transcription, whereas estradiol or tamoxifen inhibits it [45]. On the other hand, the lack of hormone efficacy in these clinical studies, in terms of tumor growth and survival, could be due to either a low expression of ER or AR in HCC or to mutated receptors expression. Therefore, further studies will be necessary to

improve patient selection on the basis of gender, ER tumor expression and their functional status. With regard to this latter aspect, considering that PgR is an estrogen inducible protein, it led us to consider that this steroid receptor could be a possible marker of ER functional status in HCC, to select candidate patients to an anti-estrogenic therapy. In addition, we demonstrated that PgR expression have a prognostic implication in patients with resected HCCs, being a factor associated with better prognostic [23], which is a clinical finding previously demonstrated in breast cancer [46-47]. This finding is very important considering also our reported positive association between PgR expression and HCV infection [23], which represents an increasing aetiology of HCC in our patient's population [3].

Several data suggest that blocking AR may be interesting in HCC therapy. Studies in animal models suggest that increased hepatic AR expression correlated with accelerated tumor development [48], or even AR expression was associated with intrahepatic recurrence in HCC [49]. However, AR expression has also been reported to be associated to small tumor size, but not with a higher rate of recurrence [28]. More recent data indicate that suppression of androgen or AR signals also led to an increase in the number of infiltrating cells, such as macrophages as well as B or T-cells [50], which shows diverse and important roles in the promotion of HCC tumorigenicity [51]. Although there is few data on the effect of anti-androgens in HCC patients, it has been suggested that anti-androgen therapy may have some benefit in patients with androgen-positive tumors [52]. Likewise, recently it has been reported that a multicenter trial with anti-androgens in HCC male patients, has been interrupted because of digestive side effects [39]. Nevertheless, further studies will be necessary to assess whether AR status may be a useful marker to select more accurately candidate patients to further anti-androgenic therapies. Notably, it has been reported that expression of androgen-induced proteins, such as apolipoprotein D or Zinc-alpha2-glycoprotein, is associated with poor prognosis in HCC.

Apolipoprotein D (ApoD) is an androgen-induced protein increased in both prostate and breast cancer cells [53-55]. A report of Utsunomiya *et al.* showed that low ApoD expression correlated significantly with less-differentiated HCCs and therefore with a worse prognosis of patients [56]. However, we did not found any relationship between ApoD expression and the hormonal receptor status in HCC [23]. Nevertheless, further studies will be also necessary in order to assess the possible value of ApoD as a biological marker of androgen response and/or other hormonal pathways in HCC. Thus, with regard to this latter aspect, although ApoD is an androgen-regulated protein, it can also be induced by other hormonal steroids or substances such as glucocorticoids, retinoic acid or 1,25-dihydroxyvitamin D₃, that might be involved in the regulation of this ApoD in human tumors [54,57-58]. This is relevant since it has been described that both retinoid and glucocorticoid receptors have been also detected in HCC [59-60], and that preliminary data show that retinoids are considered of clinical interest as cancer chemotherapeutic agents in advanced HCC [61].

Zinc-alpha2-glycoprotein (ZA2G) is also an androgen-induced protein increased in breast cancer [58,62]. Recently, ZA2G expression was found to be decreased in HCC tissues at both mRNA and protein levels. Moreover, low expression of ZA2G was notably more prevalent in

Hormone Receptor/ mRNA	Valuation parameters	Treatment	n	Clinical outcomes	Year	Reference
ER (previous patient group)	Tumor growth	Progestin	5	Tumor regression in 2	1982	[124]
NA	Anti-tumoral effect. Survival.	Tamoxifen 20 mg twice daily.	33	No effect on the tumor, in 8 remained stable between 5 and 13 months. Prolonged survival in 4 patients (over 18 months).	1990	[154]
NA	Anti-tumoral effect. Survival.	Tamoxifen 20 mg daily.	120 (placebo = 62)	No effect on the tumor. No effect on survival.	1995	[155]
Wild type and variant ER RNA m	Tumor growth.	Tamoxifen 80 mg daily in patients with wild-ERs or megestrol 160 mg daily in patients with variant ERs.	8 patients, 4 with wild ERs and 4 with variant ERs.	Tumor regression to half size in patients with wild type ERs following tamoxifen treatment. Megestrol slowed down tumor growth in tumors with variant ERs.	1996	[30]
ER y PR	Survival.	Tamoxifen.	119 (placebo = 58)	No effects on survival, regardless of the type of receptor expressed.	2000	[156]
Variant ER mRNA.	Tumor growth. Survival.	Megestrol 160 mg daily.	45 (placebo = 24).	Significantly slowed down tumor growth and improved survival in treated patients than placebo group.	2001	[157]
NA	Survival. Quality of life.	Tamoxifen 120 mg or 60 mg daily.	329	No effect on survival or on quality of life. Deleterious effects with the higher dose.	2002	[158]

ER= Estrogen receptor. NA=information not available. PR= Progesterone Receptor.

Table 4. Results of hormonal treatment in hepatocellular carcinoma

patients with poor tumor differentiation, advanced liver cirrhosis, high serum alpha fetoprotein (AFP) level and shorter survival time. These results suggest that ZA2G may be a promising novel prognostic biomarker for HCC [63], and advocate for the need for validation studies.

Recent data indicates that estrogens attenuate tumor progression in HCC *in vivo* by reducing tumor cell invasion, arresting cell cycle progression, and promoting apoptosis, which is characterized by an increased expression of cleaved caspase-3, and by a decrease in the expression of Proliferating Cell Nuclear Antigen (PCNA), cyclin A, cyclin D1, Bcl-2, and matrix metalloproteases (MMP) 2 and 9 [64]. It is relevant the data indicating that steroid hormones, such as estrogens, may inhibit MMPs in several experimental models [65-67]. This may be of great importance due to MMPs are proteolytic enzymes which participate in the degradation of the stromal connective tissue and basement membrane component, therefore facilitating tumor invasion and metastasis. Here, we show a negative relationship between ER expression and MMP2, MMP-9 or MMP-11 expression in HCC (Figure 1).

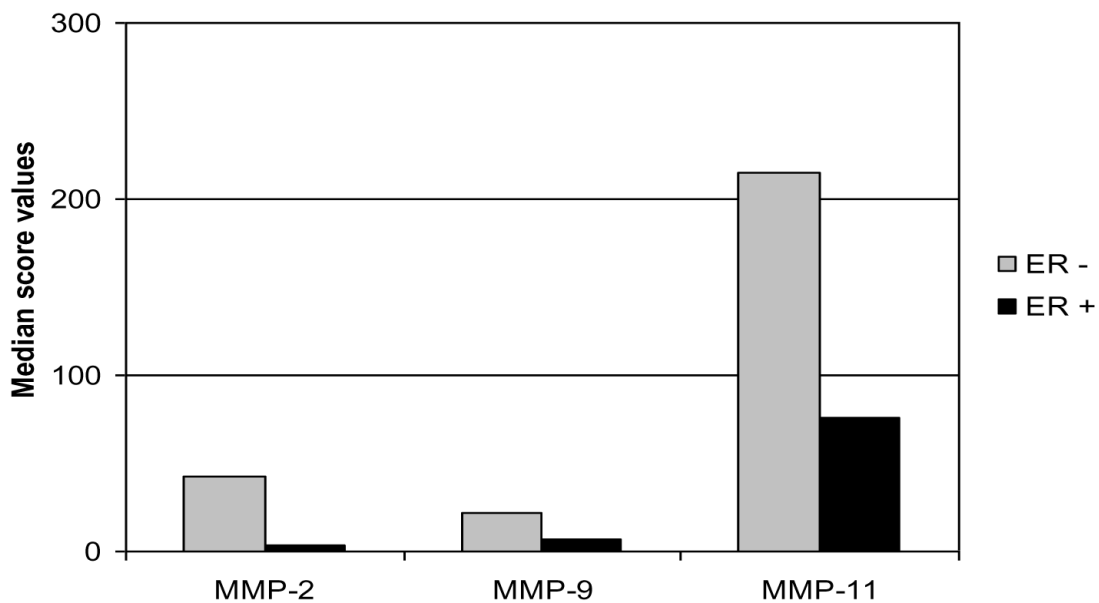


Figure 1. Relationship between ER expression and median score values of MMP2, MMP-9 or MMP-11 expression in HCC.

5. Metalloproteases and their inhibitors

Degradation of the stromal connective tissue and basement membrane components are key elements in tumor invasion and metastasis. Some components of the extracellular matrix, particularly interstitial collagens, are very resistant to proteolytic attacks, being degraded only by matrix metalloproteases (MMPs) [68].

The human MMP family currently consists of 28 members of homologous zinc-dependent endopeptidases, which can be divided into eight structural classes or, based on their substrate specificity and primary structure, into the more familiar subgroups of collagenases (MMP-1, -8 and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10, -11), membrane-associated MMPs (MMP-14, -15, -16, -17, -23, -24, -25) and other novel MMPs [69-71]. MMPs are synthe-

sized as inactive zymogens, which are then activated predominantly pericellularly by other MMPs or by serine-proteases. MMPs' activity is specifically inhibited by the so-called tissue inhibitors of metalloproteases (TIMPs). Currently, four different TIMPs are known to exist: TIMP-1, -2, -3 and -4. In addition, there are other two additional aspects conferring relevance to this enzymatic system in cancer biology. First, MMPs are able to impact on tumor cell behavior *in vivo* as a consequence of their ability to cleave growth factors, cell surface receptors, cell adhesion molecules, or chemokines/cytoquines [72-77]. Furthermore, by cleaving proapoptotic factors, MMPs may induce a more aggressive phenotype via generation of apoptotic resistant cells [78]. MMPs may also regulate angiogenesis in cancer, both positively through their ability to mobilize or activate proangiogenic factors [79-80], and negatively via generation of angiogenesis inhibitors, such as angiostatin and endostatin, cleaved from large protein precursors [81-83]. Second, it is now assumed that TIMPs are multifactorial proteins also involved in the induction of proliferation and the inhibition of apoptosis [84-85].

Previous studies have shown the expression of several MMPs in HCC [86-100]. These findings have a great interest because HCC is characterized by a disposition for vascular invasion and high metastatic potential, thus leading to high incidence of early postoperative recurrence and poor survival [101-102]. Although there is no basal membrane in the liver, HCC cancer cells grow surrounded by extracellular matrix proteins secreted as a consequence of cirrhosis, and therefore proteolytic activity is required to allow HCC cells to penetrate and cross over such tissue boundaries [103]. In fact, several studies have suggested the significance of some MMPs in the malignant behavior of HCC, such as MMP-2 [86-93], MMP-7 [88,94], MMP-9 [95-97,91,93,104], MMP-12 [105] or MMP-14 (MT1-MMP) [98,91,99-100]. In these studies, MMPs were associated with several parameters indicative of tumor aggressiveness and poor prognosis, although the cellular type expressing each factor (tumor cell and/or peritumor stromal cell) was not specifically considered.

There are few available data referring to the integrated expression of these factors in relation with HCC. In this context, we have described new findings about MMPs and TIMPs expressions in HCC, together with an important expression by tumor stromal cells, as well as their clinical relevance. It is known that MMPs expression in neoplastic tissues is high due to regulation, in a paracrine manner, by growth factors and cytokines secreted by either tumor or stromal cells [106]. Nevertheless, high MMPs and TIMPs expression by HCCs tissues may be also due to the interplay between transformed cells and their microenvironment, particularly the surrounding extracellular matrix. In human livers, fibrogenesis underlies the development of HCCs in at least 90% of cases [107], and HCC is typically surrounded by a fibrous capsule at an early stage [108]. In addition, several studies have shown that some MMPs (MMP-1, -2, -9 and -13) and TIMPs (TIMP-1 and -2) are involved in the liver fibrosis processes [90,109-112]. It has been demonstrated that extracellular matrix deposition induces overexpression of MMPs and TIMPs, such as MMP-2, secreted by human mesenchymal cells but also by hepatoma cells [87].

Recently we reported that immunostaining for MMPs and TIMPs was localized predominantly in tumor cells, but also in peritumor stromal cells in a significant percentage of cases. However,

we found no stromal expression of MMPs or TIMPs in normal liver samples [113]. We also found a positive and noteworthy association between MMP-1 expression by fibroblasts or by mononuclear inflammatory cells (MICs) and a larger tumor size. In addition, our data showed that MMP-1 expression by stromal fibroblasts, as well as the expression of MMP-13, TIMP-1 and 2, by MICs, were significantly associated with a shorter overall survival (Figure 2) [113].

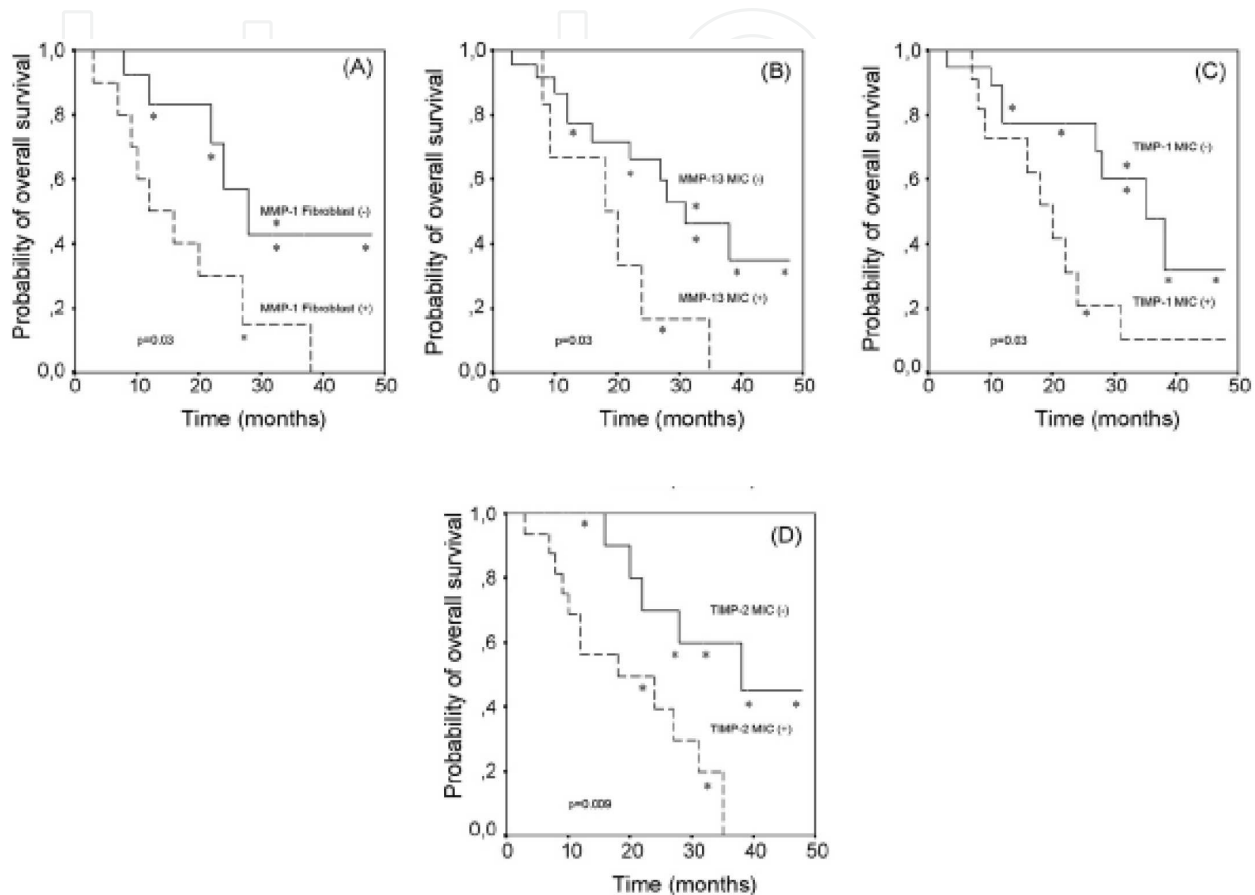


Figure 2. Kaplan-Meier survival curves as function of the expression by fibroblasts cells of MMP-1 (A); expression by MIC of MMP-13 (B), TIMP-1 (C) and TIMP-2 (D).

There are discrepancies between different studies regarding the prognostic significance of several of these factors in HCC, such as MMP-9 and MMP-2 expression in stromal compartments [113,93]. Nevertheless, it is worth considering the existence of possible differences in MMPs or TIMPs expression between different patient's populations. Thus, for example, in Asian countries HCC originates more frequently in healthy liver tissues, with less incidence of cirrhosis, than in Europe, whereas in the Mediterranean area as well as in the North American countries liver HCC only develops in chronically injured livers, where altered turn-over and increased deposition of ECM proteins has been described. In a such environment, upregulation of MMPs and TIMPs has been reported and it is possible for them to play an important role in the rearrangement of the liver tissue architecture an aspect that could influence the different expression pattern of MMPs and TIMPs in stromal HCC cells [110,114].

On the other hand, a decrease on TIMP-2 and -3 expression has been reported to be associated with invasion and metastases in HCC [115-116]. Certainly, if TIMPs inhibit MMPs *in vivo*, it should be expected that high levels of these inhibitors would prevent tumor progression and thus to be related with good outcome in cancer patients. However, TIMPs are multifunctional proteins that in addition to its MMP-inhibitory effect also shown distinct tumor-stimulatory functions, such as the induction of proliferation and the inhibition of apoptosis [84]. Thus, it is of note that TIMP-1 and -2 expressions by stromal cells were associated with shortened overall survival[113]. Accordingly, it has been reported that TIMP-1 overexpression leads to an increase of hepatoma cells migration [117], and also that it is associated with invasion and metastases in HCC [115,117].

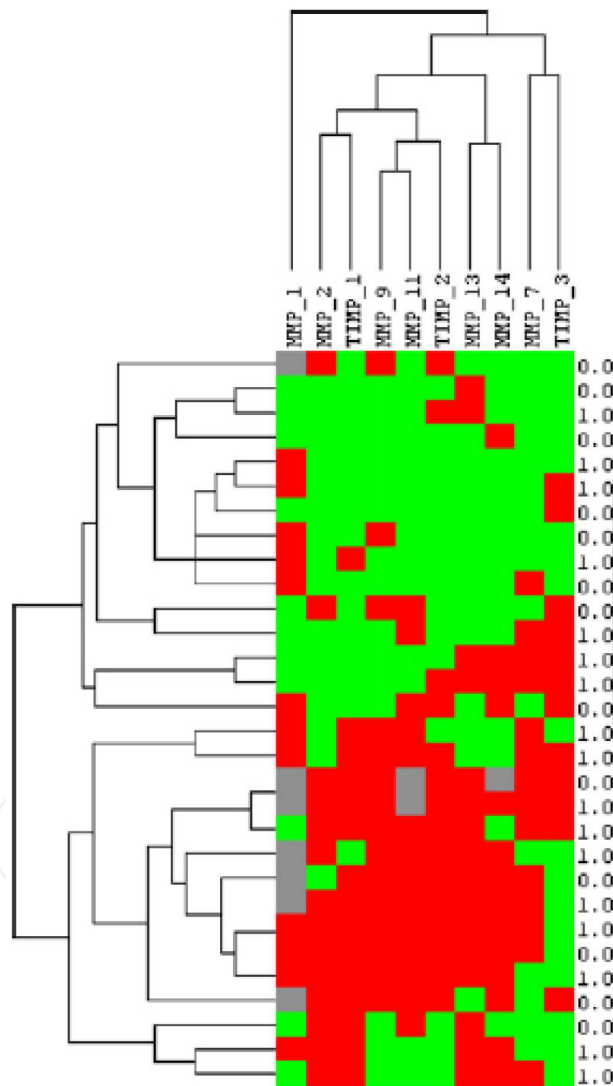


Figure 3. Graphical representation of two-dimensional unsupervised hierarchical clustering results on immunohistochemistry expression (score values) profile in 10 proteins (MMPs and TIMPs) in 30 samples of HCC tissue. Rows: tumor samples; columns: MMPs and TIMPs. Protein expressions are depicted according to a color scale: red: positive staining; green: negative staining; gray: missing data. At right bank of the figure is represented the overall survival status for each patient (0, alive; 1, death due tumor progression).

The unsupervised hierarchical cluster analysis of MMPs/TIMPs expressions in HCCs led us to identify 2 well-defined clusters of cases (Figure 3): one with low expression of MMPs and TIMPs (Group 1), and another with high expression of these factors (Group 2). This classification could have biological interest in order to identify patient candidates to new therapies based on enzymatic MMP inhibition. With regard to this, although there are no published data about use of MMP inhibitors in HCC, recently it has been reported that decreased MMP activity mediated by statins reduced progression and limits metastatic diffusion of established HCC [118]. Likewise, it was demonstrated that blocking the tumor-related glycoprotein HAb18G/CD147 by gene silence in HCC cells or with HA18 monoclonal antibody, resulted in a suppressive effect on MMP secretion and cell invasion [119]. More recent studies have shown an antimetastatic effect of Norcantharidin on HCC by transcriptional inhibition of MMP-9 [120], as well as a metastasis inhibition of HCC cells due to down-regulation of Osteopontin via a mechanism involving MMP-2 and urinary plasminogen activator (uPA) [121].

6. Conclusion

In summary, we consider that expression analysis of steroid hormone receptors, MMPs and TIMPs, contributes to a better knowledge in the biological characterization of HCC and highlights the need for further studies exploring new therapeutic targets for this common tumor.

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