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EXPRESSION OF EXTRACELLULAR MATRIX METALLOPROTEASE INDUCER (EMMPRIN) IN HEPATOCELLULAR CARCINOMA

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RESEARCH ARTICLE

ABSTRACT. HEPATOCELLULAR CARCINOMA (HCC) is one of the most frequent malignancies in the world. Clinical outcome and prognosis are still poor, likely because the occurrence of metastasis. The biological mechanisms underlying the spreading of HCC cancer cells are still unknown; however, it has been reported that increased levels of matrix metalloproteases (MMPs), a family of enzymes with a proteolytic activity towards a number of extracellular matrix (ECM) proteins, play a role in allowing HCC metastasis. CD147/EMMPRIN (extracellular matrix metalloprotease inducer), a transmembrane glycoprotein belonging to the immunoglobulin superfamily, has been shown to regulate the expression of MMPs. In this study we investigated the expression of EMMPRIN in tissues of 23 patients with metastatic and nonmetastatic HCC and in the peritumoral cirrhotic tissue. Our results show that EMMPRIN is more strongly expressed in HCC than in peritumoral tissue. In HCC patients, expression is stronger in metastatic than nonmetastatic patients. Finally, the stronger expression is inversely correlated with survival. In conclusion, EMMPRIN expression could have a role affecting HCC prognosis and survival.

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1. INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most frequent malignancy in the world; in Western and North American countries it usually develops in cirrhotic patients, likely because of the diffusion of the hepatitis C virus [1,2]. Thus, so far liver cirrhosis represents the most important risk factor in these countries [3,4]. Prognosis and survival are still unsatisfactory, mainly because of supervening metastasis occurrence [5]. No therapies are so far available to block and/or prevent HCC cancer spread, mainly because the molecular mechanisms underlying cancer cell motility are still unknown.

Matrix metalloprotease (MMP)-2 and MMP-9, are members of an enzyme family with proteolytic activity towards a number of different extra-cellular matrix (ECM) proteins, secreted as pro-enzymes and activated at the cellular surface by membrane type (MT)1-MMP complexed with the tissue inhibitor of MMP (TIMP)-2. TIMPs are molecules with a dual biological function, being involved in both the activation and the inhibition of MMPs [6,7].

It has been reported that decreased levels of TIMPs or increased levels of MMPs are responsible for a proteolytic imbalance that seems to allow cancer cell spread and the occurrence of metastasis [8,9]. Recently, CD147/EMMPRIN, a transmembrane glycoprotein belonging to the immunoglobulin superfamily, has been extensively investigated in cancer research because of its ability to stimulate the production of MMPs (such as MMP-1, MMP-2, MMP-3, MMP-9, MT1-MMP) by cancer and/or stromal cells [10,11,12]. Because of its biological functions, this molecule has been named extracellular matrix metalloprotease inducer (EMMPRIN) [13], but it is also known as M6 antigen, OX-47, HT7 antigen, Neurothelin or human basigin.

EMMPRIN is normally expressed in different human cell types such as leukocytes, red blood cells, platelets, endothelial cells [14], endometrial epithelial cells [15] and keratinocytes [16]; its expression seems to be modulated in several physiological or pathological conditions whereby an altered proteolytic balance occurs, such as in rheumatoid arthritis [17], in ventricular myocardium in heart failure [18], in liver fibrosis [19] and in venous leg ulcers [20]. EMMPRIN is highly expressed in several types of human cancers [21,22], and correlates with tumor progression and metastasis [23]. It is suggested that EMMPRIN may have a role in promoting metastasis formation in HCC, since it increases the invasive ability of hepatoma cell lines in vitro [24], while antisense RNAs show an inhibitory effect on hepatoma cells invasion [25]. We have reported that a proteolytic imbalance in HCC patients is responsible for a more aggressive and invasive phenotype leading to a worse clinical outcome and prognosis [26]. The goal of this study is to investigate the expression and the clinical relevance of CD147/EMMPRIN in patients with HCC.

2. MATERIAL AND METHODS

2.1. PATIENTS AND SPECIMENS

In this study, 23 patients with HCC (20 men, 3 women, age 42-81 years) who underwent surgery were included. After the surgical therapy, all the patients were clinically followed up for a period of 24 months in the Department of Internal Medicine, University of Bari Medical School. As shown in TABLE 1, all the patients with HCC were affected by underlying cirrhosis, which was HBV-related in

TABLE 1. CLINICAL CHARACTERISTICS OF PATIENTS WITH HCC.

Number of patients	23
Age (years, range)	42 – 81
SEX Male Female	 20 (86.9%) 3 (13.1%)
CIRRHOSIS ETIOLOGY HBV HCV Multiple viral infection Unknown	 9 10 2 2
Tumor size (cm, mean value)	6.3 ± 2.9

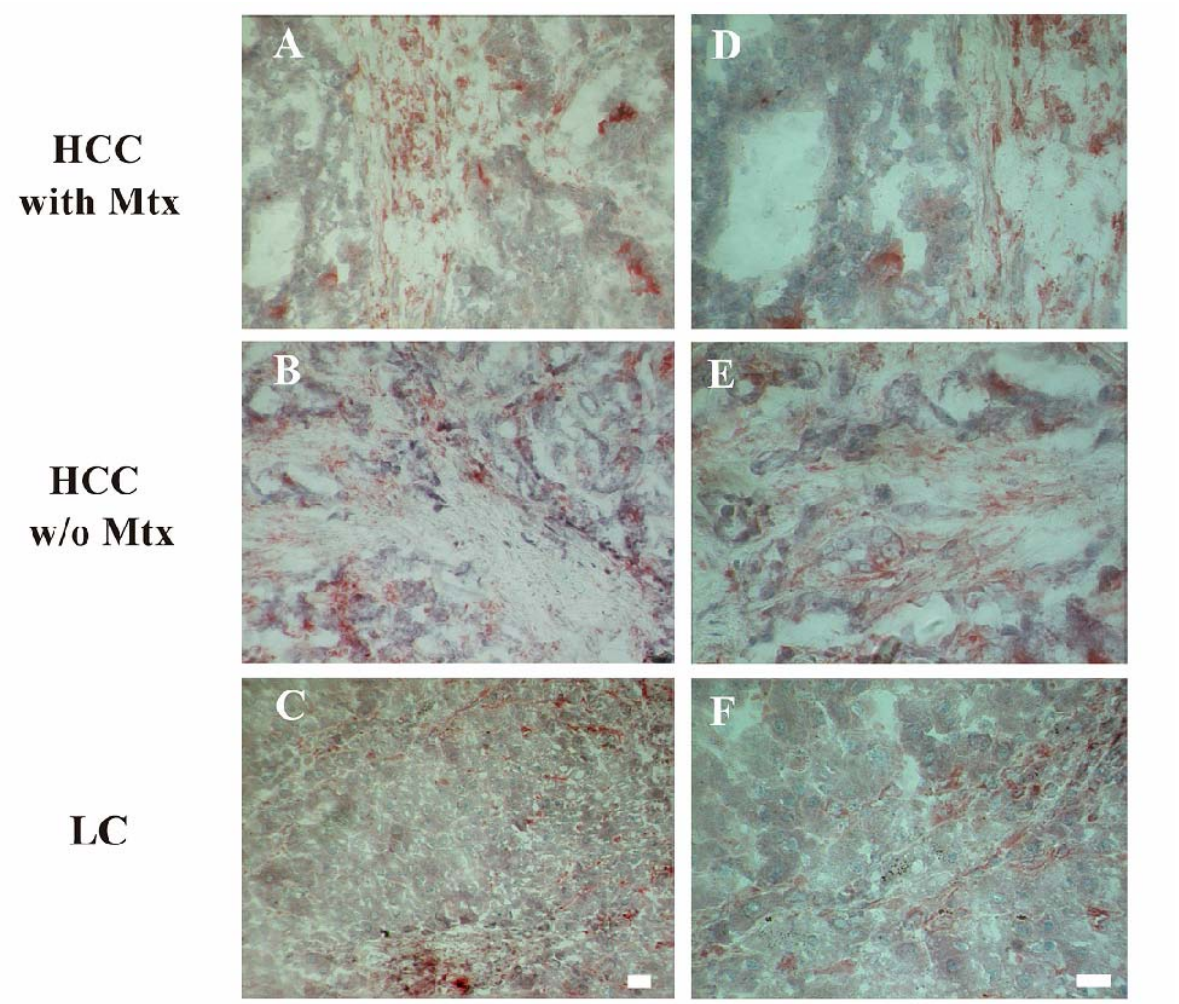

FIGURE 1. EMMPRIN EXPRESSION IN HCC AND PERITUMORAL LIVER CIRRHOSIS. EMMPRIN expression was present in the parenchyma of HCC metastatic (panel A and D) and non metastatic patients (panel B and E), while it was restricted to the stroma in the peritumoral liver cirrhosis (LC), (panel C and F). Scale bar = 20 mμ.

9/23 (39.13%), HCV-related in 10/23 (43.47%), due to multiple viral infection in 2/23 (8.69%) and of unknown etiology in 2/23 patients (8.69%).

2.2. IMMUNOHISTOCHEMISTRY

HCC tissue specimens were collected from the primary nodules and from the peritumoral area. Tissue samples were fixed with 3.7% formaldehyde and processed for routine histology, whereas adjacent specimens were immediately snap-frozen in liquid nitrogen and processed for immunohistochemistry. After embedding in Optimal Cutting Temperature (OCT) compound (Miles Laboratories Inc., Naperville, IL), serial 5 μm thick section were cut with a microtome (Microtom HM 505E, Carl Zeiss Oberkochen, Germany), collected on appropriate glass slides and processed for indirect alkaline phosphatase as previously described [26]. Briefly, sections were fixed in a cold chloroform/ acetone mixture for 10 minutes, air-dried and incubated with a polyclonal primary antibody anti-

CD147. After gentle washing, sections were incubated with a proper secondary antibody (Dako, Glostrup, Denmark) for 30 minutes in a humidified chamber. Sections were then washed and incubated with alkaline phosphatase anti-alkaline phosphatase complexes. Staining was developed with red fuchsin chromogen, and abundantly washed for 20 minutes. Finally sections were mounted with glycerol and examined with a Nikon Eclipse photomicroscope (Nikon, Corp., Tokyo, Japan).

Quantification of the staining was performed using an appropriate software, LUCIA (Nikon Corp., Tokyo, Japan) as previously described [27]. Briefly, in each patient, 10 randomly chosen microscopic fields were considered, and protein expression was measured as the mean stained area per microscopic field (± SD), calculated in square micrometers (μm^2).

2.3. STATISTICAL ANALYSIS

Student's t-test was used to determine the 99% confidence intervals (CI) for the EMMPRIN immunostaining quantification between HCC, with and without metastasis, and peritumoral liver cirrhosis tissues. The correlation between EMMPRIN expression in HCC patients with and without metastasis, and overall survival were studied with the Pearson correlation coefficient.

3. RESULTS

EMMPRIN expression was detected in 23/23 specimens of HCC tissue from the primary nodules, the distribution pattern being in the cytoplasm and at the cell-cell contact in tumoral HCC cells (FIG. 1A). It was also present at the plasma membrane level of stromal cells both in the primary HCC nodules and in the surrounding cirrhotic peritumoral tissue. However, in peritumoral tissue EMMPRIN was mainly localized in the stroma, while in HCC, both metastatic and non metastatic, it was expressed in the stroma but also in the parenchymal tissue (FIG 1B).

Immunostaining results were quantified, and, as reported in Fig.2, the expression levels of EMMPRIN in HCC primary nodules varied consid-

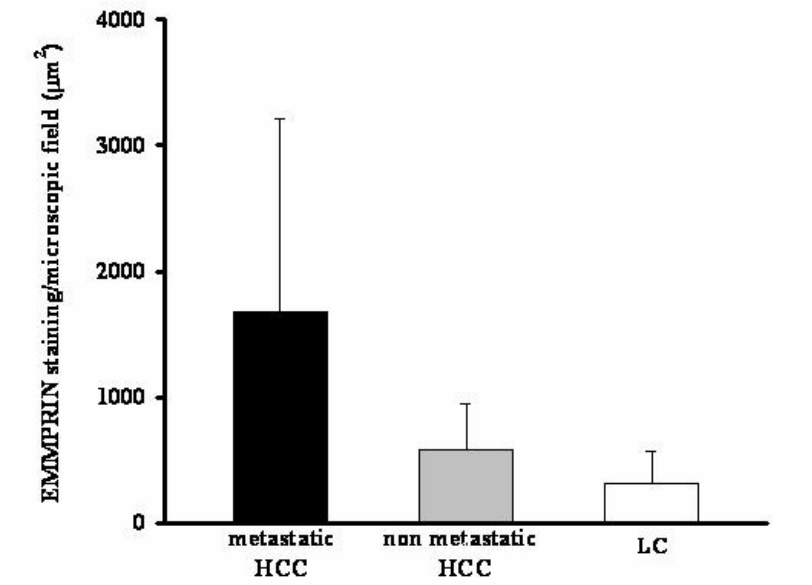

FIGURE 2. EMMPRIN EXPRESSION LEVELS IN HCC AND PERITUMORAL LIVER CIRRHOSIS. EMMPRIN expression was stronger in metastatic than non metastatic HCC. In the peritumoral liver cirrhosis (LC) EMMPRIN expression was very limited.

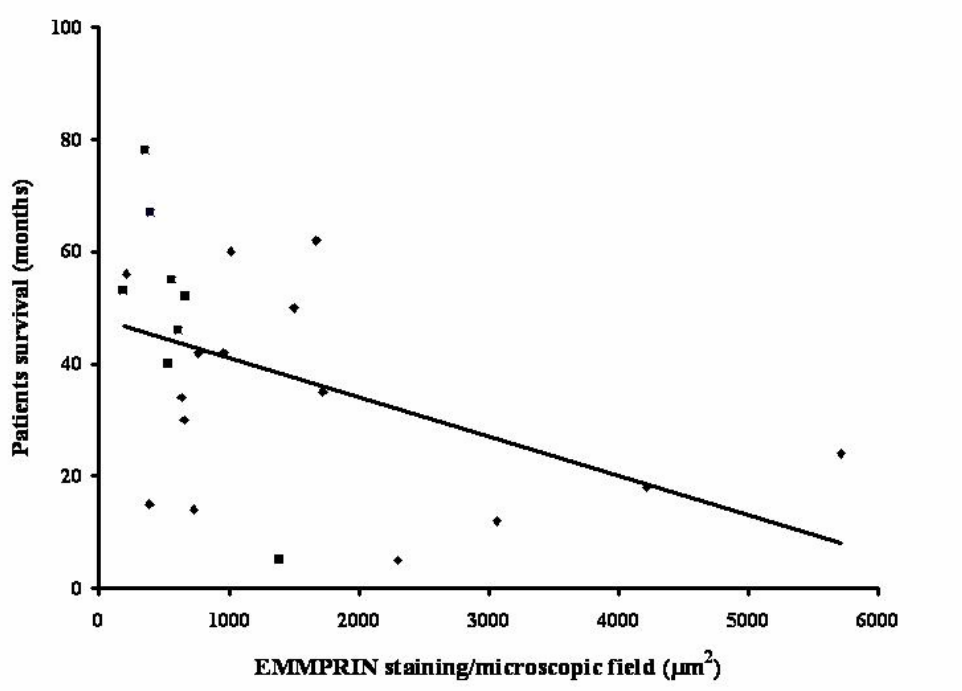

FIGURE 3. CORRELATION BETWEEN EMMPRIN EXPRESSION AND HCC PATIENTS SURVIVAL. The extent of EMMPRIN expression is inversely correlated with survival of HCC patients ($r = -0.46$; $P < 0.05$).

erably among patients, ranging from very low to very strong. In particular, in patients with a metastatic HCC the EMMPRIN expression was stronger as compared to the non metastatic patients (1673.7 ± 1537.3 and 588.1 ± 359.3 μm^2/ microscopic field, respectively), this difference being statistically significant ($P < 0.05$).

EMMPRIN expression was also detected to a lesser extent (308.7 ± 257.4) in the cirrhotic peritumoral tissue, with a statistically significant difference with respect to the HCC tissue (both from metastatic and non metastatic patients), in parenchymal and in stromal cells, with a prevalent localization at the cell-cell contact and on the cellular membranes.

A statistically significant inverse correlation ($P < 0.05$; $R = -0.460$) between EMMPRIN tissue levels in HCC primary nodules and patients survival was found, as reported in FIG. 3. Finally, analysis of the clinical outcome (FIG. 4) showed that metastatic patients had higher levels of EMMPRIN expression in HCC nodules as well as shorter survival rates when compared to the nonmetastatic patients. This difference was particularly evident at the end of the 24-month follow-up period.

4. DISCUSSION

Metastasis and disease progression in HCC are related to a shift of the proteolytic balance towards degradation [26]. However, no data are so far available concerning the mediators involved in MMPs regulation such as EMMPRIN. In this study we show that EMMPRIN is expressed in HCC primary nodules and that its expression correlates with metastasis and poor survival. This conclusion is based on the following results: (i) HCC metastatic patients show higher levels of EMMPRIN expression in HCC primary nodules as compared to non metastatic patients; (ii) overexpression of EMMPRIN in HCC primary nodules correlates with a poor clinical outcome, while patients with lower levels have longer survival.

Consistently with other studies, in our patients EMMPRIN was not exclusively expressed by can-

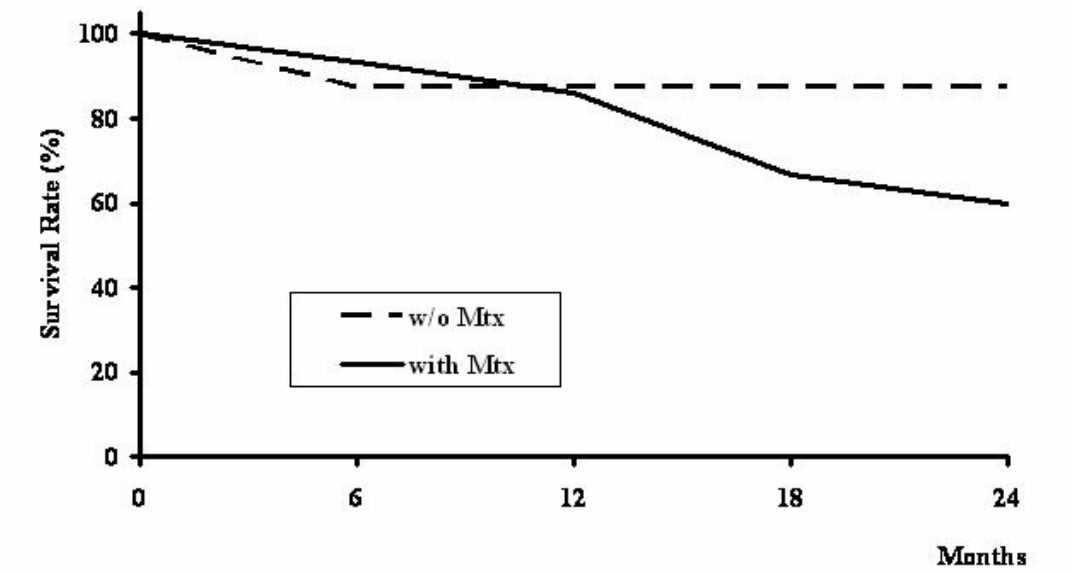

FIGURE 4. SURVIVAL OF HCC PATIENTS WITH AND WITHOUT METASTASIS. Patients with a metastatic HCC have shorter survival as compared with nonmetastatic patients.

cer cells, but also by stromal cells of both the tumoral and peritumoral tissue [23]. Thus, EMMPRIN cannot be strictly considered a marker of cancer cells, although it is overexpressed by cancer cells. These data support the idea that surrounding tissue microenvironment molecules such as EMMPRIN play a role in HCC cancer metastasis, modulating the MMPs production of cancer and/or of non-tumoral cells [28]. This is in agreement with other studies [19], reporting that EMMPRIN was also detected in the cirrhotic tissue surrounding the HCC primary nodule.

In conclusion, our data suggest that the increased tissue expression of EMMPRIN may have a role in the clinical outcome of HCC patients, likely stimulating MMP-2 and/or MMP-9 production.

REFERENCES

[1] DEUFFIC S, POYNARD T, BUFFAT L AND VALLERON AJ [1998] Trends in primary liver cancer. Lancet 351: 214-215.

[2] EL SERAG HB [2004] Hepatocellular carcinoma: recent trends in the United States. Gastroenterology 127: S27-S34.

[3] COLOMBO M [1999] Natural history and pathogenesis of hepatitis C virus related hepatocellular carcinoma. J Hepatol 31 Suppl 1: 25-30.

[4] FASANI P, SANGIOVANNI A, DE FAZIO C, BORZIO M, BRUNO S, RONCHI G, DEL NINNO E AND COLOMBO M [1999] High prevalence of multinodular hepatocellular carcinoma in patients with cirrhosis attributable to multiple risk factors. Hepatology 29: 1704-1707.

[5] GIANNELLI G, PIERRI F, TREROTOLI P, MARINOSCI F, SERIO G, SCHIRALDI O AND ANTONACI S [2002] Occurrence of portal vein tumor thrombus in hepatocellular carcinoma affects prognosis and survival. A retrospective clinical study of 150 cases. Hepatol Res 24: 50-59.

[6] GIANNELLI G AND ANTONACI S [2002] Gelatinases and their inhibitors in tumor metastasis: from biological research to medical applications. Histol Histopathol 17: 339-345.

[7] VISSE R AND NAGASE H [2003] Matrix metalloproteinases and tissue inhibitors of metalloproteinases: Structure, function, and biochemistry. Circ Res 92: 827-839.

[8] ONISTO M, RICCIO MP, SCANNAPIECO P, CAENAZZO C, GRIGGIO L, SPINA M, STETLER-STEVENSON WG AND GARBISA S [1995] Gelatinase A/TIMP-2 imbalance in lymph-node-positive breast carcinomas, as measured by RT-PCR. Int J Cancer 63: 621-626.

[9] FATA JE, WERB Z AND BISSELL MJ [2004] Regulation of mammary gland branching morphogenesis by the extracellular matrix and its remodeling enzymes. Breast Cancer Res 6: 1-11.

[10] KATAOKA H, DECASTRO R, ZUCKER S AND BISWAS C [1993] Tumor cell-derived collagenase-stimulatory factor increases expression of interstitial collagenase, stromelysin, and 72-kDa gelatinase. Cancer Res 53: 3154-3158.

[11] LIM M, MARTINEZ T, JABLONS D, CAMERON R, GUO H, TOOLE B, LI JD AND BASBAUM C [1998] Tumor-derived EMMPRIN (extracellular matrix metalloproteinase inducer) stimulates collagenase transcription through MAPK p38. FEBS Lett 441: 88-92.

[12] SAMESHIMA T, NABESHIMA K, TOOLE BP, YOKOGAMI K, OKADA Y, GOYA T, KOONO M AND WAKISAKA S [2000] Glioma cell extracellular matrix metalloproteinase inducer (EMMPRIN) (CD147) stimulates production of membrane-type matrix metalloproteinases and activated gelatinase A in co-cultures with brain-derived fibroblasts. Cancer Lett 157: 177-184.

[13] BISWAS C, ZHANG Y, DECASTRO R, GUO H, NAKAMURA T, KATAOKA H AND NABESHIMA K [1995] The human tumor cell-derived collagenase stimulatory factor (renamed EMMPRIN) is a member of the immunoglobulin superfamily. Cancer Res 55: 434-439.

[14] KASINRERK W, TOKRASINWIT N AND PHUNPAE P [1999] CD147 monoclonal antibodies induce homotypic cell aggregation of monocytic cell line U937 via LFA-1/ICAM-1 pathway. Immunology 96: 184-192.

[15] NOGUCHI Y, SATO T, HIRATA M, HARA T, OHAMA K AND ITO A [2003] Identification and characterization of extracellular matrix metalloproteinase inducer in human endometrium during the menstrual cycle in vivo and in vitro. J Clin Endocrinol Metab 88: 6063-6072.

[16] BORDADOR LC, LI X, TOOLE B, CHEN B, REGEZI J, ZARDI L, HU Y AND RAMOS DM [2000] Expression of emmprin by oral squamous cell carcinoma. Int J Cancer 85: 347-352.

[17] TOMITA T, NAKASE T, KANEKO M, SHI K, TAKAHI K, OCHI T AND YOSHIKAWA H [2002] Expression of extracellular matrix metalloproteinase inducer and enhancement of the production of matrix metalloproteinases in rheumatoid arthritis. Arthritis Rheum 46: 373-378.

[18] SPINALE FG, COKER ML, HEUNG LJ, BOND BR, GUNASINGHE HR, ETOH T, GOLDBERG AT, ZELLNER JL AND CRUMBLEY AJ [2000] A matrix metalloproteinase induction/activation system exists in the human left ventricular myocardium and is upregulated in heart failure. Circulation 102: 1944-1949.

[19] SHACKEL NA, MCGUINNESS PH, ABBOTT CA, GORRELL MD AND MCCAUGHAN GW [2002] Insights into the pathobiology of hepatitis C virus-associated cirrhosis: analysis of intrahepatic differential gene expression. Am J Pathol 160: 641-654.

[20] NORGAUER J, HILDENBRAND T, IDZKO M, PANTHER E, BANDEMIR E, HARTMANN M, VANSCHEIDT W AND HEROUY Y [2002] Elevated expression of extracellular matrix metalloproteinase inducer (CD147) and membrane-type matrix metalloproteinases in venous leg ulcers. Br J Dermatol 147: 1180-1186.

[21] VAN DEN OORD JJ, PAEMEN L, OPDENAKKER G AND WOLF-PEETERS C [1997] Expression of gelatinase B and the extracellular matrix metalloproteinase inducer EMMPRIN in benign and malignant pigment cell lesions of the skin. Am J Pathol 151: 665-670.

[22] MURAOKA K, NABESHIMA K, MURAYAMA T, BISWAS C AND KOONO M [1993] Enhanced expression of a tumor-cell-derived collagenase-stimulatory factor in urothelial carcinoma: its usefulness as a tumor marker for bladder cancers. Int J Cancer 55: 19-26.

[23] DAVIDSON B, GIVANT-HORWITZ V, LAZAROVICI P, RISBERG B, NESLAND JM, TROPE CG, SCHAEFER E AND REICH R [2003] Matrix metalloproteinases (MMP), EMMPRIN (extracellular matrix metalloproteinase inducer) and mitogen-activated protein kinases (MAPK): co-expression in metastatic serous ovarian carcinoma. Clin Exp Metastasis 20: 621-631.

[24] JIANG JL, ZHOU Q, YU MK, HO LS, CHEN ZN AND CHAN HC [2001] The involvement of HAb18G/CD147 in regulation of store-operated calcium entry and metastasis of human hepatoma cells. J Biol Chem 276: 46870-46877.

[25] LI Y, SHANG P, QIAN AR, WANG L, YANG Y AND CHEN ZN [2003] Inhibitory effects of antisense RNA of HAb18G/CD147 on invasion of hepatocellular carcinoma cells in vitro. World J Gastroenterol 9: 2174-2177.

[26] GIANNELLI G, BERGAMINI C, MARINOSCI F, FRANSVEA E, QUARANTA M, LUPO L, SCHIRALDI O AND ANTONACI S [2002] Clinical role of MMP-2/TIMP-2 imbalance in hepatocellular carcinoma. Int J Cancer 97: 425-431.

[27] GIANNELLI G, MARINISCI F, SGARRA C, LUPO L, DENTICO P AND ANTONACI S. [2005] Clinical role of tissue and serum levels of SCCA antigen in hepatocellular carcinoma. Int J Cancer [in press].

[28] QUARANTA V AND GIANNELLI G [2003] Cancer invasion: watch your neighbourhood! Tumori 89: 343-348.

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