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• REVIEW •

The prognostic molecular markers in hepatocellular carcinoma

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Abstract

The prognosis of hepatocellular carcinoma (HCC) still remains dismal, although many advances in its clinical study have been made. It is important for tumor control to identify the factors that predispose patients to death. With new discoveries in cancer biology, the pathological and biological prognostic factors of HCC have been studied quite extensively. Analyzing molecular markers (biomarkers) with prognostic significance is a complementary method. A large number of molecular factors have been shown to associate with the invasiveness of HCC, and have potential prognostic significance. One important aspect is the analysis of molecular markers for the cellular malignancy phenotype. These include alterations in DNA ploidy, cellular proliferation markers (PCNA, Ki-67, Mcm2, MIB1, MIA, and CSE1L/CAS protein), nuclear morphology, the p53 gene and its related molecule MDM2, other cell cycle regulators (cyclin A, cyclin D, cyclin E, cdc2, p27, p73), oncogenes and their receptors (such as ras, c-myc, c-fms, HGF, c-met, and erb-B receptor family members), apoptosis related factors (Fas and FasL), as well as telomerase activity. Another important aspect is the analysis of molecular markers involved in the process of cancer invasion and metastasis. Adhesion molecules (E-cadherin, catenins, serum intercellular adhesion molecule-1, CD44 variants), proteinases involved in the degradation of extracellular matrix (MMP-2, MMP-9, uPA, uPAR, PAI), as well as other molecules have been regarded as biomarkers for the malignant phenotype of HCC, and are related to prognosis and therapeutic outcomes. Tumor angiogenesis is critical to both the growth and metastasis of cancers including HCC, and has drawn much attention in recent years. Many angiogenesis-related markers, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived endothelial cell growth factor (PD-ECGF), thrombospondin (TSP), angiogenin, pleiotrophin, and endostatin (ES) levels, as well as inratumor microvessel density (MVD) have been evaluated and found to be of prognostic significance. Body fluid (particularly blood and urinary) testing for biomarkers is easily accessible and useful in clinical patients. The prognostic significance of circulating DNA in plasma or serum, and its genetic alterations in HCC are other important trends. More attention should be paid to these two areas in future. As the progress of the human genome project advances, so does a clearer understanding of tumor biology, and mo re and more new prognostic markers with high sensitivity and specificity will be found and used in clinical assays. However, the combination of some items, i. e., the pathological features and some biomarkers mentioned above, seems to be mor e practical for now.

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INTRODUCTION

Liver cancer is one of the common malignancies worldwide, and has been ranked the 2nd cancer killer in China since the 1990s. Although many advances in the clin ical study of hepatocellular carcinoma (HCC) have been made, and long-term surv ival of patients has been obtained in some clinical centers, only a definitive s ubset of cases is cured by surgery, and the overall dismal outcome of patients w ith HCC has not been completely changed. Lack of control of metastatic foci and recurrence are the most prevalent causes of death in patients with HCC, and it is important for tumor control to identify the factors that predispose patients to death. Much effort has been made to predict HCC behavior, but specific prognos tic indicators are still lacking[1].

Prognostic factors in HCC conventionally consist of staging with the tumor node metastasis system (TNM) and grading by tumor cellular differentiation. There are also other factors useful in prognostic predication but most of them are clinic al. With new discoveries in cancer biology, pathological and biological factors of HCC in relation to prognosis have been studied quite extensively. Morphologic al features of the tumor, both gross and histological, have been found to signif icantly associate with tumor recurrence and patient survival[2-4]. A complementary way is to analyze molecular markers for their prognostic significance with reference to tumor recurrence and survival term in HCC. A large number of molecular biological factors have been shown to associate with the invasiveness of HCC, and have potential prognostic significance. However, routine biomarkers for the prediction of HCC prognosis are not yet available. In this review, we will focus on the recent advances in this aspect.

Cellular malignancy is a very important aspect for patient prognosis. In recent years, with the development of cellular and molecular biological techniques, many molecular markers related to invasion, metastasis, recurrence and survival hav e been explored. In HCC, DNA ploidy, the proliferating activity of tumor cells, tumor suppressor and promoter genes, cell cycle controllers, proteinases that d egrade extracellular matrix, adhesion molecules, angiogenic factors, and metabol ic genes, have been regarded as biomarkers for the malignant phenotype of HCC, and are related to prognosis and therapeutic outcomes[5].

CELLUAR MALIGNANCY-RELATED MARKERS

DNA-ploidy

Controversy still exists regarding the prognostic significance of DNA ploidy in HCC patients. Many reports indicate that DNA ploidy could be a predictive marker for HCC prognosis[6,7]. The overall survival rate of patients with aneuploid cells is much lower than that of patients with diploid ones, and those wit h multiple G0/G1 peaks have the

worst prognosis. Patients with higher cell proportions in proliferating stages have a higher early recurrence rate[7]. However, other studies could not find a relationship between DNA ploidy and prognosis.

Proliferating activity of HCC cells

Many antigens, such as proliferating cell nuclear antigen (PCNA), Ki-67, Mcm2, MIB1, MIA, and CSE1L/CAS protein (CAS), have been used as proliferation markers for cancer cells. The detection of PCNA with immunohistochemical methods is a co mmon way to study the proliferating activity of cancer cells[8]. Combine d with histopathological characteristics, the PCNA labeling index (PCNA-LI) is one useful marker for evaluating malignant grade, and for predicting recurrence time and the patients' prognosis of HCC[9].

The expression of the human Ki-67 protein is strictly associated with cell prol iferation. The fact that the Ki-67 protein is present during all active phases of the cell cycle, but is absent from resting cells, makes it an excellent marke r for determining the so-called growth fraction of a given cell population. Ki -67 protein expression is an absolute requirement for progression through the cell-division cycle. The fraction of Ki-67-positive tumor cells (the Ki-67 la beling index) is often correlated with the clinical course of the disease[10,11]. Higher Ki-67 labeling index (Ki-67-LI) has a very similar clinical significance to PCNA-LI, reflecting the existence of biologically aggressive ph enotypes and poor overall and disease-free survival rates in HCC. This could be a useful factor for predicting the long-term survival of patients with HCC fol lowing hepatic resection[12,13].

The CSE1L/CAS protein (CAS) is a Ran-binding protein with a function as a nuclear transport (export) factor. This protein plays a role in the mitotic spindle checkpoint, which assures genomic stability during cell division. This checkpoint is frequently disturbed in neoplasias of various origins, including hepatic tum ors. The degree of CAS expression correlates with the grade of tumor dedifferent iation, and could be a prognostic marker for HCC[14].

Nuclear morphology

Nuclear profiles have been reported as useful prognostic predictors in various c ancers, including HCC. The nuclear area of HCC correlates with cell differentiation and cell proliferating activity, and HCC with a large nuclear area has high potential for blood vessel invasion and intrahepatic metastasis. Computerized nu clear morphometry is more objective and quicker than conventional microscopic an alysis[15]. Recently, quantitative nuclear morphometry of cancer cells followed by computer-assisted image analysis (termed Quantitative nuclear grade, QNG) has proven to have potential use in cancer detection and predicting outcomes such as tumor stage, recurrence, and progression[16].

p53 gene and its related molecule MDM2

P53 protein plays a central role in cellular responses, including cell-cycle ar rest and cell death in response to DNA damage. p53 dysfunction can induce abnorm al cell growth, increased cell survival, genetic instability, and drug resistanc e. Mutations in the p53 gene are the most frequently reported somatic gene alter ation in human cancer. Associations of p53 mutation or positive immunohistochemi stry staining with higher grade and more advanced stage has been noted for cance rs of various origins. In addition, p53 mutation is considered as a strong marke r predicting an increased risk of local relapse, treatment failure, and overall and disease-free survival in many kinds of human carcinomas, such as breast[17-19], colorectal[20], esophageal[21], head and neck[22], lung[23,24], and ovarian[25], as well as sarcoma[26]. An increased intracellular concentration of the P53 protein, although no t identical to, is sometimes seen in tumors with p53 mutation, and has been corr elated with poor prognosis in some tumor types. Several studies have shown a rel ationship between the nuclear accumulation of p53 protein and poor disease-free and overall survival of cancer patients[27,28]. The presence of serum a nti-p53 antibody has also been shown to associate with survival of patients wit h breast, ovarian, and colorectal cancer[29,30]. p53 mutations in plasma DNA could also be detected in cancer patients, and may be used as a prognostic factor and an early marker to indicate recurrence or distant metastasis[31]. However, there is still a great controversy as to whether alteration of th e p53 gene adversely affects survival of cancer patients. Many reports failed to show the independent prognostic value of p53 in the carcinomas of tongue[32], breast[33,34], stomach[35], lung[36], ovarian[37], bladder[38], colorectal[39], and non-Hodgkin's lympho ma[40].

In a similar situation, there are many very controversial results with the progn ostic value of p53 overexpression or p53 gene mutation in HCC patients. Many stu dies showed that p53 mutation was involved in determining the dedifferentiation, the proliferating activity, and tumor progression[41], was strongly rel ated to the invasiveness of HCC, and may also influence the postoperative course (particularly the recurrence within 1 year)[42,43]. Mutations in the p53 gene or positive immunostaining for mutant P53 protein expression could be use d as a significant indicator of poor prognosis. HCCs with p53 mutations have a h igh malignant potential, and p53 mutation in the primary lesion is useful as an indicator for the biological behavior of recurrent HCCs. It is also a useful ind ependent prognostic factor affecting survival after recurrence[9,44,45].

In a recent prospective study, we found the 3-year and 5-year overall surviva l rates of HCC patients with positive P53 nuclear accumulation were much lower than those of the HCC patients with negative P53 expression. In univariate and mu ltivariate Cox analysis, p53 overexpression was the most significant factor that associated with the overall survival rates of HCC patients after resection. Its significance was even greater than that of factors such as tumor size, vascular invasion, and tumor capsule, though they were also related to the overall survi val. p53 mutation or nuclear accumulation of p53 expression could be a valuable marker for predicting the prognosis of HCC patients after resection[46].

Serum anti-p53 antibody also could be a useful prognostic factor for HCC patien ts[47]. However, many different results showed that neither the immunohi stochemical detection of p53 expression, nor the serum anti-p53 antibodies had a significant prognostic value for outcome of patients with HCC[48,49].

The transcription of the mdm2 gene is activated by p53 and this limits the growt h-suppressing activity of p53 by direct binding. It has been reported that MDM2 protein is overexpressed in several types of cancers. Endo found MDM2 overexpre ssion correlated positively with p53 mutation, and is a useful predictor of poor prognosis in patients with HCC following hepatic resection[50].

Cell cycle regulators

Disruption of the G1/S and G2/M check points leads to uncontrolled cell growth, resulting in the development and progression of cancers. Overexpression of cycli n A, cyclin D, and cyclin E have been found to correlate with the tumor relapse of human HCC, and are independent predictive markers for their recurrence and prognosis[51,52]. The enhanced expression of cyclin E correlates with hype rphosphorylation of pRb and a high frequency of Ki-67-positive cells. HCCs wit h enhanced cyclin E expression probably contain a relatively large number of pro liferating cancer cells[52]. cdc2 overexpression seems to play the most crucial role of the modulators in cell cycle progression and cell proliferation of HCC, and significantly predicts recurrence[53].

The p27 protein binds and inhibits cyclin/cyclin-dependent kinase complexes, is a negative regulator of cell-cycle progression. The central role of p27 makes it important in a variety of disease processes, particularly in neoplasia, that involve aberrations in cellular proliferation and other cell fates. Loss of p27 cooperates with mutations in several oncogenes and tumor suppressor genes to facilitate

tumor growth, indicating that p27 may be a "nodal point" for tumor suppression. In most tumor types, reduced p27 expression correlates with poor prog nosis, making p27 a novel and powerful prognostic marker[54]. High p27 e xpression, correlated with prolonged survival, is a favorable independent progno stic parameter for HCC[55,56].

The protein p73, the first identified homologue of p53 gene, has been shown to i nduce apoptosis. P73 expression status is significantly related to prognosis of HCC patients, and could serve as a useful indicator of prognosis in HCC patients [57]. There is still controversy with the prognostic value of the p16IN K4a and p15INK4B genes[58].

Tumor promoter genes and their receptors

Aberrations of many tumor promoter genes, such as ras, c-myc, c-fms have been indicated as indicators of malignant potential and poor prognosis i n HCC[9,59-61]. c-myc amplification and p53 alteration may be copa rticipating events in the progression of HCC. Disease-free survival in patients showing c-myc amplification is significantly shorter than in those without amplification.Hepatocyte growth factor/scatter factor (HGF/SF) is one of the most important hu moral mediators of liver regeneration. It is potentially related to molecular me chanisms of hepatocarcinogenesis via a paracrine system involving its cellular r eceptor, c-met. Up-regulation of c-met plays an important role in the development and progression of HCC, and may be a prognostic marker. Its express ion level is inversely correlated with survival coordinated with uPA expression [62,63]. However, there is no significant correlation between the HGF le vel in tumor and the survival rate of HCC patients[5].

Among of the erb-B receptor family members, c-erbB-2 (Her-2/neu) represents a well-established prognostic marker and therapeutic target in several human tu mor types, especially breast cancer. However, c-erbB-2 is neither a prognostic marker nor a relevant therapeutic target in human HCCs[9,64]. EGF-R and c-erbB-3 play important roles in the progression of HCC, affecting disease-free survival of HCC patients[5,65].

ets-1 has also been shown to link to cancer invasion and metastasis. ets-1 exp ression was observed with high incidence. However, the average labeling index (L I) in HCC is lower than in noncancerous lesions. Even lower expression levels we re found in HCCs of high TNM stage, poor differentiation, portal invasion, intra hepatic metastasis, large tumor size, and high Ki-67-LI. HCC patients with hig h ets-1 expression showed better outcomes for disease-free survival than those with low ets-1 expression[66].

Apoptosis related

The expression of Fas and Fas ligand (Fas L) play a role in apoptosis of cancer cells including HCCs, and associates with the prognosis of cancer patients. Fas expression level is significantly decreased in poorly differentiated HCC and of large size, while Fas L expression in carcinoma cells is observed exclusively in moderately or poorly differentiated cases. Each of them has prognostic signific ance for disease-free survival (DFS)[5,67,68].

Telomerase activity

The ribonucleoprotein telomerase extends telomeres in cancer cells and has been proposed as a prognostic marker for cancer. Telomerase activity can be identified as an independent predictor for recurrence after resection of HCC[69]. The peripheral blood telomerase activity can also be used as a molecular marker for the detection of circulating hepatoma cells in blood of HCC patients, which reflect haematogenous micrometastasis. This is potentially a practical diagnostic/predictive marker of HCC[70]. Quantitative analysis of telomerase act ivity shows that the patients with positive telomerase activity in noncancerous liver tissue have a higher recurrence rate after HCC resection. The relative tel omerase activity (RTA) of early recurrent patients is significantly higher than those without recurrence. So, RTA could be a predictive marker for early recurre nce after HCC resection[71].

CELL ADHESION AND EXTRACELLULAR MATRIX RELATED

Adhesion molecules

The expression level of E-cadherin inversely correlates with HCC histological g rade and prognosis. E-cadherin underexpression might have some contribution to the early recurrence of HCC[72,73]. In contrast, alpha-, beta-, and ga mma-catenin expression significantly correlated positively with HCC grade, bein g the highest in poorly differentiated HCC. Significant positive associations were found between gamma-catenin high expression and capsular invasion or presence of satellite nodules, and between beta-catenin high expression and vascular i nvasion. HCC patients with underexpression of E-cadherin, alpha-catenin, and gamma-catenin, and patients with overexpression of beta-catenin, had poorer sur vival rates[73]. HCCs with a nonnuclear type of beta-catenin overexpres sion were frequently larger than 5cm in diameter and had poorer cellular diff erentiation, more invasiveness, and the patients had significantly shorter disea se-free survival lengths[74,75]. beta-catenin mutation associat es with nuclear expression of the protein, and is a favorable prognostic factor related to low stage[76].

Serum concentration of intercellular adhesion molecule-1 (sICAM-1) in patients with HCC is a marker for disease progression and prognosis. Higher sICAM-1 lev els are more frequently observed in those patients with multiple lesions and int rahepatic metastasis, and their prognosis is also very poor. Detecting sICAM-1 is of important value in predicting tumor recurrence after surgery[77-79]. The CD44 proteins form a ubiquitously expressed family of cell surface adhesion molecules involved in cell-cell and cell-matrix interactions. The major physio logical role of CD44 is to maintain organ and tissue structure via cell-cell an d cell-matrix adhesion, but certain variant isoforms can also mediate lymphocyte activation and homing, and the presentation of chemical factors and hormones. The expression of multiple CD44 isoforms is greatly upregulated in neoplasia. CD 44, particularly its variants, may be useful as a diagnostic or prognostic marker of malignancy in at least some human cancers[80]. Up-regulation of CD 44 isoforms such as CD44s, CD44v5, CD44v6, CD44v7-8, and CD44v10, correlates with high histological grade, being the highest in poorly differentiated HCC. CD44 s positivity was an independent factor. Positivity for one or more CD44 isoforms was the most useful independent factor for overall survival[81].

Degradation of extracellular matrix

The matrix metalloproteinases (MMP) and the plasminogen activation system (PA) p lay crucial roles in the process of cancer invasion and metastasis. Their expres sion levels were found correlated to recurrence and survival after HCC resection[82,83].

MMP-2, MMP-9, and tissue inhibitors of metalloproteinases -1, -2 (TIMP-1, T IMP-2) have been found to be of prognostic significance in HCC. The content of MMP-2, MMP-9 in HCC being higher than that in surrounding liver parenchyma could be used as an important index to judge the invasion and metastasis of HCC [84,85]. Plasma MMP-9 levels can also be a candidate for a novel marker for HCC. The levels appear to reflect the potential and ongoing activity of vascula r invasion[5].

In several tumor types, elevated levels of urokinase plasminogen activator (uPA) , its receptor (uPAR) or its inhibitor plasminogen activator inhibitor-1 (PAI-1) is associated with a poorer prognosis[85]. uPA activity may be the mo st sensitive factor affecting HCC invasion in the plasminogen activation system and is a strong predictor for the recurrence and prognosis of HCC[86,87]. The PAI-1 proteinis a multifaceted proteolytic factor. It not only functions as an inhibitor of the protease uPA, but also plays an important role in signal

transduction, cell adherence, and cell migration. Thus,an apparent paradox cons idering its name-although it inhibits uPA during blood coagulation, it actually promotes invasion and metastasis. In many malignancies including HCC, elevated PAI-1 is associated with tumor aggressiveness and poor patient outcome[86].

ANGIOGENESIS RELATED

Tumor angiogenesis is critical to both the growth and metastasis of cancer, and is regulated by angiogenic factors. Circulating angiogenesis regulators have bee n evaluated not only as diagnostic and/or prognostic factors but also as predict ive factors in cancer patients. They could be used to determine the risk of deve loping cancer, to screen for early detection, to distinguish benign from malignant disease, and to distinguish between different types of malignancies. In estab lished malignances, they can be uses to determine prognosis, to predict the resp onse to therapy, and to monitor the clinical course[5,83,88]. HCC is typically a hypervascular tumor with a rich blood supply. In recent years, many angiogenesis-related markers, such as vascular endothelial growth factor (VEGF), b asic fibroblast growth factor (bFGF), platelet-deriwed endothelial cell growth factor (PD-ECGF), thrombospondin (TSP), angiogenin, pleiotrophin and endostatin (ES) levels, as well as inratumor microvessel density (MVD) have been evaluated and found to relate to HCC prognosis.

Intratumor microvessel density (MVD)

The intratumor MVD is a direct reflection of tumor angiogenesis. It can be visua lized by immunohistochemical staining with antibodies to anti-CD34, Factor VIII, and alpha smooth muscle actin[89]. MVD levels have a close relationshi p with the tumor capsule status, tumor size (HCC with 2-5cm in diameter has t he highest MVD level), intrahepatic recurrence and disease-free survival, and can be a predictive marker for disease-free survival[90]. In the authors ' institute, three types of intratumor microvessels, including capillary-like, sinusoid-like, and mixed-type, were found in HCC. The MVD level was not related to tumor size, capsule status, Edmondson's grade, or alpha-fetoprotein level; was an independent factor of disease-free survival in small HCC patients; and was a predictive marker for early recurrence[91].

Vascular endothelial growth factor (VEGF)

A substantial number of studies have demonstrated a strong association between elevated tumor expression of VEGF and advanced disease or poor prognosis in vario us cancers. Circulating VEGF seems to be a reliable surrogate marker of angiogen ic activity and tumor progression in cancer patients. It may be predictive of tumor status and prognosis in patients with different types of cancer, and may be useful in predicting and monitoring tumor response to anticancer therapies and i n follow-up!surveillance for tumor relapse. It may provide new prognostic infor mation that is not afforded by conventional clinicopathologic prognostic indicat ors[92]. In HCC, a high serum VEGF level significantly correlates with a bsence of tumor capsule, presence of intrahepatic metastasis, presence of micros copic venous invasion, and advanced stage, and it may be useful as a biologic ma rker of tumor invasiveness and a prognostic factor in HCC[93]. The data of the authors' institute also shows serum VEGF is a predictor of invasion and m etastasis of HCC and a potential biomarker of metastatic recurrence after curati ve resection[94,95].

Platelet-derived endothelial cell growth factor (PD-ECGF)

PD-ECGF may not be a major regulator of angiogenesis of HCC, but may play an im portant role in hepatocarcinogenesis, cooperating with hepatitis C virus. PD-EC GF expression associates with the venous invasion of HCC[96].

GENOMICS AND PROTEOMICS RELATED

Molecular genetic analyses have clarified that accumulation of genomic changes p rovides important steps in carcinogenesis and have identified a number of valuab le genetic markers for certain cancers. The association of these genomic aberrat ions with the progression and prognosis of cancer has drawn more and more attent ion. To date, allelic loss of 1p, 1q21-23, 2p21-16.3, 3p24-p25, 8p22, 8p23, 9 p21, 9q, 10, 13q12, 17p13.3, and 22q13 have been proposed to be related to the s urvival and prognosis of cancer patients[97-101].

Many chromosomal aberrations, including gain of 1q, 8q, and 20q, and loss of 16q , 4q, 17p, 1p, and 8p have been identified in HCC[102]. However, the rel ationship between these recurrent alterations and the clinical phenotypes and pr ognosis is still unknown. Towards the end of 1999, we compared the differences of chromosomal aberrations between the primary HCC tumors and their matched metas tatic lesions using a comparative genomic hybridization (CGH) technique, and fou nd chromosome 8p deletions might contribute to HCC metastasis[103]. This result was further confirmed by comparison between nude mice models of HCC with different metastatic potentials[104]. In addition, a more accurate loca tion was identified on 8p23.3, 8p11.2[105]. These findings provide new targets for exploring new predictive markers for the recurrence and prognosis of HCC. Recently, Itano *et al*. used restriction landmark genomic scanning (RLGS), a new high-speed screening method for multiple genomic changes, to detect un known genetic alterations in HCC. They found the disease-free survival r ate for patients with > or =16 changed RLGS spots was significantly lower than that for patients with fewer changed RLGS spots (<or =15 spots). In multivariate analysis, the number of changed spots was proven to retain an independent progno stic value. These results suggest that the number of changed RLGS spots may be a useful biological marker for recurrence of HCC[106].

One important trend in this area that should be paid attention to is the prognos tic value of circulating DNA in plasma or serum, and its genetic alterations in cancer patients. Small amounts of DNA circulate in both healthy and diseased hum an plasma/serum, and increased concentrations of DNA are present in the plasma of cancer patients. Characteristics of tumor DNA have been found in genetic mater ial extracted from the plasma of cancer patients. These features include decreas ed strand stability, the presence of specific oncogene or tumor suppressor gene mutations, microsatellite alterations, Ig rearrangements and hypermethylation of several genes. The results obtained in many different cancers have opened a new research area indicating that plasma DNA might eventually be a suitable target for the development of noninvasive diagnostic, prognostic and follow-up tests for cancer[107]. Blood testing for circulating tumor genetic markers may provide valuable prognostic information and guide future therapy[108].

However, there is still controversy over the prognostic significance [109]. We found loss of heterozygosity (LOH) on chromosome 14q (D14S62 and D14S51) could be detected in plasma DNA, and could be of prognostic significance in HCC patients[1].

Proteomics, regarded as a sister technology to genomics, is one of the technologies rapidly changing our approach to understanding tumor biology. By com paring the proteins present in diseased samples with those present in normal sam ples, it is possible to identify changes in expression of proteins that potentia lly may be related to tumor progression, invasion and metastasis, and prognosis. This technique has now made it possible to analyze proteins using high th roughput, automated techniques.Proteomic profiling can be applied to tissue sam ples as well as body fluids (e.g.serum,urine,etc.),and it can provide surrogate markers of disease processes,potential response to treatment, possibility of recu rrence and metastasis for cancers including HCC[110].

OTHERS

In addition, higher levels of urinary TGF-beta 1[111], heat shock prote in-27 (HSP-27)[112] and Glutamine synthetase (GS) expression

in the tu mor[113], increased levels of cyclooxygenase-2 (COX-2) in nontumor liv er tissue[114], preoperative serum IL-10[115], and HFE mutation s[116] or down-regulation of DRH1[117] are also powerful progno stic indicators for shorter disease-free survival and poor prognosis, related t o tumor progression of HCC.

The RECK (reversion-inducing-cysteine-rich protein with Kazal motifs) gene suppresses the invasive and metastatic activities of cancers, has negative effects on the invasiveness of HCC, and can be regarded as a promising prognostic molec ular marker for HCC[118].

EXPERIENCES OF THE AUTHORS' INSTITUTE

At the authors' institute, many molecular factors have been investigated and fou nd to be related to HCC invasiveness in recent years. They could be divided into two groups: one is positive invasiveness-related factors, including p16 and p53 mutations, H-ras, c-erbB2, mdm2, TGF-a, epidermal growth factor receptor (E GFR), MMP-2, uPA, uPA-R and PAI-1, ICAM-1, VEGF, PD-ECGF, bFGF, and osteopo ntin (OPN), etc. The other group is negative invasiveness-related factors, incl uding nm23-H1, Kai-1, TIMP-2, integrin a5, E-cadherin, etc. These factors co uld be potential predictive markers for the prognosis of HCC. Serum ICAM-1 and PAI-1 levels were higher in patients with metastasis than those without metast asis, while serum Thrombomodulin concentration negatively associated with the in trahepatic spreading and portal vein thrombi of HCC. Deletions of chromosome 8p and 17p, overexpression of MMP-2, TGF-a, and EGFR in HCC tissues, and LOH on c hromosome 14q (D14S62 and D14S51) in plasma DNA were also related to metastatic recurrence and prognosis of HCC patients. p53 mutation or nuclear accumulation of p53 expression could be a valuable marker for predicting the prognosis of HCC patients after resection. E-cadherin, nm23, TIMP-2 are promising prognostic ma rkers[1, 43,46,77,84,86,91,94-96,104-106].

To search for metastasis-associated genes on a global genomic scale, we recently used cDNA microarrays containing approximately 9984 human transcripts to inves tigate the gene expression profiles of primary tumors and their corresponding metastatic lesions (intrahepatic metastasis or tumor thrombosis of portal vein). A total of 79 significantly upregulated and 69 downregulated genes were identified. Some of them have proven to promote HCC metastasis[119]. These will p rovide new prognostic markers for predicting the possibillity of metastatic recu rrence and survival after operation.

QUESTIONS AND PROSPECTS

In summary, pathologic factors indicative of tumor invasiveness such as tumor size, number, capsule state, venous invasion, presence of satellite nodules, and advanced pTNM stage, are the best-established risk factors for recurrence and im portant aspects affecting the prognosis of patients with HCC. Recent molecular r esearch has identified many tumor biological factors as potential prognostic mar kers (biomarkers). However, to date, none of them has been proved to be specific enough, and most of the studies for specific molecular parameters were correlat ive and retrospective. Methodologies, sample sizes, and definitions differ. Cons ideration should be given to the design of prospective clinical trials in evalua ting the prognostic significance of these markers.

These biomarkers could be detected both in tissue and body fluids (serum, urine, bile, etc.). Body fluid (particularly blood and urinary) testing is easily acce ssible and useful in clinical patients, and is more important in "predicting" the possibility or "early diagnosis" of recurrence and metastasis. So, future work should be focused on serum or urinary markers.

The prognostic significance of circulating DNA in plasma or sesum, and its genet ic alterations in HCC, are important trends that deserve attention. Proteomics and cDNA array provide other ways to explore new prognostic markers. So, we can b elieve, with the continuing progress of human genome project, the development of new molecular and cytogenetic techniques, and a more complete understanding of tumor biology, more and more new prognostic markers with high sensitivity and specificity will soon be found and used in clinical assays. However, the combination of some items, i.e., pathological features and some biomarkers mention ed above, seems to be more practical now.

REFERENCES

1 Tang ZY. Hepatocellular carcinoma-Cause, treatment and metastasis. *World J Gastroenterol* 2001;7:445-454
2 Qin LX, Tang ZY. The prognostic significance of clinical and pathological features in hepatocellular carcinoma. *World J Gastroenterol* 2002;8:193-199
3 Zhao WH, Ma ZM, Zhou XR, Feng YZ, Fang BS. Prediction of recurrence and prognosis in patients with hepatocellular carcinoma after resection by use of CL IP score. *World J Gastroenterol* 2002;8:237-242
4 Zheng N, Ye SL, Sun RX, Zhao Y, Tang ZY. Effects of cryopreservation and phenylacetate on biological characters of adherent LAK cells from patients with hepatocellular carcinoma. *World J Gastroenterol* 2002;8:233-236
5 Korn WM. Moving toward an understanding of the metastatic process in hep atocellular carcinoma. *World J Gastroenterol* 2001; 7:777-778
6 Mise K, Tashiro S, Yogita S, Wada D, Harada M, Fukuda Y, Miyake H, Isika wa M, Izumi K, Sano N. Assessment of the biological malignancy of hepatocellular carcinoma: relationship to clinicopathological factors and prognosis. *Clin Ca ncer Res* 1998;4:1475-1482
7 Nolte M, Werner M, Nasarek A, Bektas H, von Wasielewski R, Klempnauer J, Georgii A. Expression of proliferation associated antigens and detection of num erical chromosome aberrations in primary human liver tumours: relevance to tumou r characteristics and prognosis. *J Clin Pathol* 1998; 51:47-51
8 Weber JC, Nakano H, Bachellier P, Oussoultzoglou E, Inoue K, Shimura H, Wolf P, Chenard-Neu MP, Jaeck D. Is a proliferation index of cancer cells a rel iable prognostic factor after hepatectomy in patients with colorectal liver meta stases? *Am J Surg* 2001;182:81-88
9 Lin GY, Chen ZL, Lu CM, Li Y, Ping XJ, Huang R. Immunohistochemical stud y on p53, H-rasp21, c-erbB-2 protein and PCNA expression in HCC tissues of Ha n and minority ethnic patients. *World J Gastroenterol* 2000;6: 234-238
10 Scholzen T, Gerdes J. The Ki-67 protein: from the known and t he unknown. *J Cell Physiol* 2000;182:311-322
11 Hernandez-Rodriguez NA, Correa E, Sotelo R, Contreras-Paredes A, Gom ez-Ruiz C, Green L, Mohar A. Ki-67: a proliferative marker that may predict pu lmonary metastases and mortality of primary osteosarcoma. *Cancer Detect Prev* 2001; 25:210-215
12 Ito Y, Matsuura N, Sakon M, Takeda T, Umeshita K, Nagano H, Nakamori S , Dono K, Tsujimoto M, Nakahara M, Nakao K, Monden M. Both cell proliferation an d apoptosis significantly predict shortened disease-free survival in hepatocell ular carcinoma. *Br J Cancer* 1999;81: 747-751
13 Ouchi K, Sugawara T, Ono H, Fujiya T, Kamiyama Y, Kakugawa Y, Mikuni J , Yamanami H, Komatsu S, Horikoshi A. Mitotic index is the best predictive facto r for survival of patients with resected hepatocellular carcinoma. *Dig Surg* 2000; 17:42-48
14 Wellmann A, Flemming P, Behrens P, Wuppermann K, Lang H, Oldhafer K, P astan I, Brinkmann U. High expression of the proliferation and apoptosis associa ted CSE1L/CAS gene in hepatitis and liver neoplasms: correlation with tumor prog ression. *Int J Mol Med* 2001;7: 489-494
15 Ikeguchi M, Sato N, Hirooka Y, Kaibara N. Computerized nuclear morphom etry of hepatocellular carcinoma and its relation to proliferative activity. *J Surg Oncol* 1998;68: 225-230
16 Veltri RW, Partin AW, Miller MC. Quantitative nuclear grade

(QNG): a n ew image analysis-based biomarker of clinically relevant nuclear structure alte rations. *J Cell Biochem* 2000;Suppl 35:151-157
17 Overgaard J, Yilmaz M, Guldberg P, Hansen LL, Alsner J. TP53 mutation is an independent prognostic marker for poor outcome in both node-negative and node-positive breast cancer. *Acta Oncol* 2000;39:327-333
18 Takahashi M, Tonoki H, Tada M, Kashiwazaki H, Furuuchi K, Hamada J, Fu jioka Y, Sato Y, Takahashi H, Todo S, Sakuragi N, Moriuchi T. Distinct prognosti c values of p53 mutations and loss of estrogen receptor and their cumulative eff ect in primary breast cancers. *Int J Cancer* 2000;89: 92-99
19 Blaszyk H, Hartmann A, Cunningham JM, Schaid D, Wold LE, Kovach JS, So mmer SS. A prospective trial of midwest breast cancer patients: a p53 gene mutat ion is the most important predictor of adverse outcome. *Int J Cancer* 2000; 89 :32-38
20 Kahlenberg MS, Stoler DL, Rodriguez-Bigas MA, Weber TK, Driscoll DL, Anderson GR, Petrelli NJ. p53 tumor suppressor gene mutations predict decreased survival of patients with sporadic colorectal carcinoma. *Cancer* 2000;88: 1814 -1819
21 Ireland AP, Shibata DK, Chandrasoma P, Lord RV, Peters JH, DeMeester T R. Clinical significance of p53 mutations in adenocarcinoma of the esophagus and cardia. *Ann Surg* 2000; 231:179-187
22 Tamas L, Kraxner H, Mechtler L, Repassy G, Ribari O, Hirschberg A, Szentkuti G, Jaray B, Szentirmay Z. Prognostic significance of P53 histochem istry and DNA histogram parameters in head and neck malignancies. *Anticancer Res* 2000; 20:4031-4037
23 Murakami I, Hiyama K, Ishioka S, Yamakido M, Kasagi F, Yokosaki Y. p53 gene mutations are associated with shortened survival in patients with advanced non-small cell lung cancer: an analysis of medically managed patients. *Clin Cancer Res* 2000;6:526-530
24 Mitsudomi T, Hamajima N, Ogawa M, Takahashi T. Prognostic significance of p53 alterations in patients with non-small cell lung cancer: a meta-analys is. *Clin Cancer Res* 2000; 6:4055-4063
25 Shahin MS, Hughes JH, Sood AK, Buller RE. The prognostic significance of p53 tumor suppressor gene alterations in ovarian carcinoma. *Cancer* 2000;8 9:2006-2017
26 de Alava E, Antonescu CR, Panizo A, Leung D, Meyers PA, Huvos A G, Pardo-Mindan FJ, Healey JH, Ladanyi M. Prognostic impact of P53 status in Ew ing sarcoma. *Cancer* 2000;89: 783-792
27 Leibovich BC, Cheng L, Weaver AL, Myers RP, Bostwick DG. Outcome predi ction with p53 immunostaining after radical prostatectomy in patients with local ly advanced prostate cancer. *J Urol* 2000;163:1756-1760
28 Osaki T, Kimura T, Tatemoto Y, Dapeng L, Yoneda K, Yamamoto T. Diffuse mode of tumor cell invasion and expression of mutant p53 protein but not of p21 protein are correlated with treatment failure in oral carcinomas and their meta static foci. *Oncology* 2000;59:36-43
29 Suzuki M, Ohwada M, Saga Y, Kohno T, Takei Y, Sato I. Micrometastatic p53-positive cells in the lymph nodes of early stage epithelial ovarian cancer: prognostic significance. *Oncology* 2001;60:170-175
30 Shiota G, Ishida M, Noguchi N, Oyama K, Takano Y, Okubo M, Katayama S, Tomie Y, Harada K, Hori K, Ashida K, Kishimoto Y, Hosoda A, Suou T, Kanbe T, Ta naka K, Nosaka K, Tanida O, Kojo H, Miura K, Ito H, Kaibara N, Kawasaki H. Circulating p53 antibody in patients with colorectal cancer: relation to clinicopatho logic features and survival. *Dig Dis Sci* 2000;45:122-128
31 Shao ZM, Wu J, Shen ZZ, Nguyen M. p53 mutation in plasma DNA and its p rognostic value in breast cancer patients. *Clin Cancer Res* 2001;7:2222-2227
32 Kantola S, Parikka M, Jokinen K, Hyrynkangs K, Soini Y, Alho OP, Salo T. Prognostic factors in tongue cancer - relsative importance of demographic, c linical and histopathological factors. *Br J Cancer* 2000;83:614-619
33 Ferrero JM, Ramaioli A, Formento JL, Francoual M, Etienne MC, Peyrotte s I, Ettore F, Leblanc-Talent P, Namer M, Milano G. P53 determination alongside classical prognostic factors in node-negative breast cancer: an evaluation at more than 10-year follow-up. *Ann Oncol* 2000;11:393-397
34 Reed W, Hannisdal E, Boehler PJ, Gundersen S, Host H, Marthin J. The prognostic value of p53 and c-erb B-2 immunostaining is overrated for pa tients with lymph node negative breast carcinoma: a multivariate analysis of pro gnostic factors in 613 patients with a follow-up of 14-30 years. *Cancer* 20 00;88: 804-813
35 Kaye PV, Radebold K, Isaacs S, Dent DM. Expression of p53 and p21waf1/ cip1 in gastric carcinoma: lack of inter-relationship or correlation with progn osis. *Eur J Surg Oncol* 2000;26: 39-43
36 Schiller JH, Adak S, Feins RH, Keller SM, Fry WA, Livingston RB, Hammo nd ME, Wolf B, Sabatini L, Jett J, Kohman L, Johnson DH. Lack of prognostic sign ificance of p53 and K-ras mutations in primary resected non-small-cell lung c ancer on E4592: a Laboratory Ancillary Study on an Eastern Cooperative Oncology Group Prospective Randomized Trial of Postoperative Adjuvant Therapy. *J Clin Oncol* 2001; 19:448-457
37 Gadducci A, Cianci C, Cosio S, Carnino F, Fanucchi A, Buttitta F, Cont e PF, Genazzani AR. p53 status is neither a predictive nor a prognostic variable in patients with advanced ovarian cancer treated with a paclitaxel-based regim en. *Anticancer Res* 2000;20:4793-4799
38 Fleshner N, Kapusta L, Ezer D, Herschorn S, Klotz L. p53 nuclear accum ulation is not associated with decreased disease-free survival in patients with node positive transitional cell carcinoma of the bladder. *J Urol* 2000;164:1 177-1182
39 Gallego MG, Acenero MJ, Ortega S, Delgado AA, Cantero JL. Prognostic i nfluence of p53 nuclear overexpression in colorectal carcinoma. *Colon Rectum* 2000;43:971-975
40 Nieder C, Petersen S, Petersen C, Thames HD. The challenge of p53 as p rognostic and predictive factor in Hodgkin's or non-Hodgkin's lymphoma. *Ann H ematol* 2001;80:2-8
41 Itoh T, Shiro T, Seki T, Nakagawa T, Wakabayashi M, Inoue K, Okamura A . Relationship between p53 overexpression and the proliferative activity in hepa tocellular carcinoma. *Int J Mol Med* 2000;6:137-142
42 Jeng KS, Sheen IS, Chen BF, Wu JY. Is the p53 gene mutation of prognos tic value in hepatocellular carcinoma after resection? *Arch Surg* 2000;135:13 29-1333
43 Tang ZY, Qin LX, Wang XM, Zhou G, Liao Y, Weng Y, Jiang XP, Lin ZY, Li u KD, Ye SL. Alterations of oncogenes, tumor suppressor genes and growth factors in hepatocellular carcinoma: with relation to tumor size and invasiveness. *Chin Med J* 1998; 111:313-318
44 Sugo H, Takamori S, Kojima K, Beppu T, Futagawa S. The significance of p53 mutations as an indicator of the biological behavior of recurrent hepatocel lular carcinomas. *Surg Today* 1999;29:849-855
45 Heinze T, Jonas S, Karsten A, Neuhaus P. Determination of the oncogene s p53 and C-erb B2 in the tumour cytosols of advanced hepatocellular carcinoma (HCC) and correlation to survival time. *Anticancer Res* 1999;19:2501-2503
46 Qin LX, Tang ZY, Ma ZC, Wu ZQ, Zhou XD, Ye QH, Ji Y, Huang LW, Jia HL, Sun HC, Wang L. P53 immunohistochemistry scoring is an independent prognostic m arker of patient with hepatocellular carcinoma resection: A prospective study of 256 formalin-fixed paraffin-embedded tumor samples. *World J Gastroenterol* 2002(in press)
47 Shiota G, Kishimoto Y, Suyama A, Okubo M, Katayama S, Harada K, Ishida M, Hori K, Suou T, Kawasaki H. Prognostic significance of serum anti-p53 antib ody in patients with hepatocellular carcinoma. *J Hepatol* 1997;27:661-668
48 Tangkijvanich P, Janchai A, Charuruks N, Kullavanijaya P, Theamboonler s A, Hirsch P, Poovorawan Y. Clinical associations and prognostic significance o f serum anti-p53 antibodies in Thai patients with hepatocellular carcinoma. *A sian Pac J Allergy Immunol* 2000;18:237-243
49 Saffroy R, Lelong JC, Azoulay D, Salvucci M, Reynes M, Bismuth H, Debu ire B, Lemoine A. Clinical significance of circulating anti-p53 antibodies in E uropean patients with

hepatocellular carcinoma. *Br J Cancer* 1999;79:604-610
50 Endo K, Ueda T, Ohta T, Terada T. Protein expression of MDM2 and its c linicopathological relationships in human hepatocellular carcinoma. *Liver* 2000;20:209-215
51 Chao Y, Shih YL, Chiu JH, Chau GY, Lui WY, Yang WK, Lee SD, Huang TS. Overexpression of cyclin A but not Skp 2 correlates with the tumor relapse of hu man hepatocellular carcinoma. *Cancer Res* 1998;58:985-990
52 Ohashi R, Gao C, Miyazaki M, Hamazaki K, Tsuji T, Inoue Y, Uemura T, H irai R, Shimizu N, Namba M. Enhanced expression of cyclin E and cyclin A in huma n hepatocellular carcinomas. *Anticancer Res* 2001;21:657-662
53 Ito Y, Takeda T, Sakon M, Monden M, Tsujimoto M, Matsuura N. Expressio n and prognostic role of cyclin-dependent kinase 1 (cdc2) in hepatocellular car cinoma. *Oncology* 2000;59: 68-74
54 Philipp-Staheli J, Payne SR, Kemp CJ. p27(Kip1): regulation and funct ion of a haploinsufficient tumor suppressor and its misregulation in cancer. *Exp Cell Res* 2001;264: 148-168
55 Fiorentino M, Altimari A, D'Errico A, Cukor B, Barozzi C, Loda M, Grig ioni WF. Acquired expression of p27 is a favorable prognostic indicator in patie nts with hepatocellular carcinoma. *Clin Cancer Res* 2000;6:3966-3972
56 Ito Y, Matsuura N, Sakon M, Miyoshi E, Noda K, Takeda T, Umeshita K, N agano H, Nakamori S, Dono K, Tsujimoto M, Nakahara M, Nakao K, Taniguchi N, Mond en M. Expression and prognostic roles of the G1-S modulators in hepatocellular carcinoma: p27 independently predicts the recurrence. *Hepatology* 1999;30:90 -99
57 Tannapfel A, Wasner M, Krause K, Geissler F, Katalinic A, Hauss J, Mossner J, Engeland K, Wittekind C. Expression of p73 and its relation to histopath ology and prognosis in hepatocellular carcinoma. *J Natl Cancer Inst* 1999; 91: 1154-1158
58 Qin Y, Li B, Tan YS, Sun ZL, Zuo FQ, Sun ZF. Polymorphism of p16INK4a gene and rare mutation of p15INK4b gene exon2 in primary hepatocarcinoma. *World J Gastroenterol* 2000;6:411-414
59 Wang Q, Lin ZY, Feng XL. Alterations in metastatic properties of hepat ocellular carcinoma cell following H-ras oncogene transfection. *World J Gastr oenterol* 2001;7:335-339
60 Cui J, Yang DH, Bi XJ, Fan ZR. Methylation status of c-fms oncogenein HCC and its relationship with clinical pathology. *World J Gastroenterol* 200 1;7:136-139
61 Kawate S, Fukusato T, Ohwada S, Watanuki A, Morishita Y. Amplification of c-myc in hepatocellular carcinoma: correlation with clinicopathologic f eatures, proliferative activity and p53 overexpression. *Oncology* 1999;57:157 -163
62 Tavian D, De Petro G, Benetti A, Portolani N, Giulini SM, Barlati S. u -PA and c-MET mRNA expression is co-ordinately enhanced while hepatocyte grow th factor mRNA is down-regulated in human hepatocellular carcinoma. *Int J Cancer* 2000; 87:644-649
63 Luo YQ, Wu MC, Cong WM. Gene expression of hepatocyte growth factor an d its receptor in HCC and nontumorous liver tissues. *World J Gastroenterol* 1 999;5:119-121
64 Prange W, Schirmacher P. Absence of therapeutically relevant c-erbB- 2 expression in human hepatocellular carcinomas. *Oncol Rep* 2001;8:727-730
65 Ito Y, Takeda T, Sakon M, Tsujimoto M, Higashiyama S, Noda K, Miyoshi E, Monden M, Matsuura N. Expression and clinical significance of erb-B receptor family in hepatocellular carcinoma. *Br J Cancer* 2001;84:1377-1383
66 Ito Y, Miyoshi E, Takeda T, Sakon M, Noda K, Tsujimoto M, Monden M, Ta niguchi N, Matsuura N. Expression and possible role of ets-1 in hepatocellular carcinoma. *Am J Clin Pathol* 2000;114:719-725
67 Ito Y, Monden M, Takeda T, Eguchi H, Umeshita K, Nagano H, Nakamori S, Dono K, Sakon M, Nakamura M, Tsujimoto M, Nakahara M, Nakao K, Yokosaki Y, Mats uura N. The status of Fas and Fas ligand expression can predict recurrence of hepatocellular carcinoma. *Br J Cancer* 2000;82:1211-1217
68 Wang XZ, Chen XC, Yang YH, Chen ZX, Huang YH, Tao QM. Relationship bet ween HBxAg and Fas/FasL in patients with hepatocellular carcinoma. *World J Gas troenterol* 2000;6:S17
69 Kobayashi T, Kubota K, Takayama T, Makuuchi M. Telomerase activity as a predictive marker for recurrence of hepatocellular carcinoma after hepatectomy. *Am J Surg* 2001; 181:284-288
70 Tatsuma T, Goto S, Kitano S, Lin YC, Lee CM, Chen CL. Telomerase activ ity in peripheral blood for diagnosis of hepatoma. *J Gastroenterol Hepatol* 2 000;15:1064-1070
71 Suda T, Isokawa O, Aoyagi Y, Nomoto M, Tsukada K, Shimizu T, Su zuki Y, Naito A, Igarashi H, Yanagi M, Takahashi T, Asakura H. Quantitation of t elomerase activity in hepatocellular carcinoma: a possible aid for a prediction of recurrent diseases in the remnant liver. *Hepatology* 1998;27: 402-406
72 Huang GT, Lee HS, Chen CH, Sheu JC, Chiou LL, Chen DS. Correlation of E-cadherin expression and recurrence of hepatocellular carcinoma. *Hepatogastr oenterology* 1999;46:1923-1927
73 Endo K, Ueda T, Ueyama J, Ohta T, Terada T. Immunoreactive E-cadherin , alpha-catenin, beta-catenin, and gamma-catenin proteins in hepatocellular c arcinoma: relationships with tumor grade, clinicopathologic parameters, and pati ents' survival. *Hum Pathol* 2000; 31:558-565
74 Wong CM, Fan ST, Ng IO. beta-Catenin mutation and overexpression in h epatocellular carcinoma: clinicopathologic and prognostic significance. *Cancer* 2001;92:136-145
75 Cui J, Zhou XD, Liu YK, Tang ZY, Zile MH. Abnormal Cu-catenin gene ex pression with invasiveness of primary hepatocellular carcinoma in China. *World J Gastroenterol* 2001;7: 542-546
76 Hsu HC, Jeng YM, Mao TL, Chu JS, Lai PL, Peng SY. Beta-catenin mutati ons are associated with a subset of low-stage hepatocellular carcinoma negative for hepatitis B virus and with favorable prognosis. *Am J Pathol* 2000;157:76 3-770
77 Sun JJ, Zhou XD, Liu YK, Tang ZY, Feng JX, Zhou G. Xue Q, Chen J. Inva sion and metastasis of liver cancer: expression of intercellular adhesion molecu le-1. *J Cancer Res Clin Oncol* 1999; 125:28-34
78 Mei MH, Xu J, Shi QF, Yang JH, Chen Q, Qin LL. Clinical significance o f serum intercellular adhesion molecule-1 detection in patients with hepatocell ular carcinoma. *World J Gastroenterol* 2000;6:408-410
79 Xu J, Mei MH, Zeng SE, Shi QF, Liu YM, Qin LL. Expressions of ICAMa21 and its mRNA in sera and tissues of patients with hepatocellular carcinoma. *World J Gastroenterol* 2001;7:120-125
80 Goodison S, Urquidi V, Tarin D. CD44 cell adhesion molecules. *Mol Pathol* 1999;52:189-196
81 Endo K, Terada T. Protein expression of CD44 (standard and variant iso forms) in hepatocellular carcinoma: relationships with tumor grade, clinicopatho logic parameters, p53 expression, and patient survival. *J Hepatol* 2000;32:78 -84
82 Sakamoto Y, Mafune K, Mori M, Shiraishi T, Imamura H, Mori M, Takayama T, Makuuchi M. Overexpression of MMP-9 correlates with growth of small hepatoc ellular carcinoma. *Int J Oncol* 2000;17:237-243
83 Jiang YF, Yang ZH, Hu JQ. Recurrence or metastasis of HCC: predictors, early detection and experimental antiangiogenic therapy. *World J Gastroenterol* 2000;6:61-65
84 Bu W, Tang ZY, Ye SL, Liu KD, Huang XW, Gao DM. The association of type IV collagenase with invasion and metastasis of hepatocellular carcinoma . *Zhonghua Xiaohua Zazhi* 1999; 19:13-15
85 Fox SB, Taylor M, Grondahl-Hansen J, Kakolyris S, Gatter KC, H arris AL. Plasminogen activator inhibitor-1 as a measure of vascular remodelli ng in breast cancer. *J Pathol* 2001; 195:236-243
86 Zheng Q, Tang ZY, Xue Q, Shi DR, Song HY, Tang HB. Invasion and metast asis of hepatocellular carcinoma in relation to urokinase-type plasminogen acti vator, its receptor and inhibitor. *J Cancer Res Clin Oncol* 2000;126:641-646
87 Itoh T, Hayashi Y, Kanamaru T, Morita Y, Suzuki S, Wang W,

Zhou L, Rui JA, Yamamoto M, Kuroda Y, Itoh H. Clinical significance of urokinase-ty pe plasminogen activator activity in hepatocellular carcinoma. *J Gastroenterol Hepatol* 2000;15: 422-430
88 Kuroi K, Toi M. Circulating angiogenesis regulators in cancer patients . *Int J Biol Markers* 2001;16:5-26
89 Morinaga S, Imada T, Shimizu A, Akaike M, Sugimasa Y, Takemiya S, Taka nashi Y. Angiogenesis in hepatocellular carcinoma as evaluated by alpha smooth m uscle actin immuno histochemistry. *Hepatogastroenterology* 2001;48:224-228
90 El-Assal ON, Yamanoi A, Soda Y, Yamaguchi M, Igarashi M, Yamamoto A, Nabika T, Nagasue N. Clinical significance of microvessel density and vascular e ndothelial growth factor expression in hepatocellular carcinoma and surrounding liver: possible involvement of vascular endothelial growth factor in the angioge nesis of cirrhotic liver. *Hepatology* 1998;27:1554-1562
91 Sun HC, Tang ZY, Li XM, Zhou YN, Sun BR, Ma ZC. Microvessel density of hepatocellular carcinoma: its relationship with prognosis. *J Cancer Res Clin Oncol* 1999;125:419-26
92 Poon RT, Fan ST, Wong J. Clinical implications of circulating angiogen ic factors in cancer patients. *J Clin Oncol* 2001;19: 1207-1225
93 Poon RT, Ng IO, Lau C, Zhu LX, Yu WC, Lo CM, Fan ST, Wong J. Serum vas cular endothelial growth factor predicts venous invasion in hepatocellular carci noma: a prospective study. *Ann Surg* 2001;233:227-235
94 Li XM, Tang ZY, Qin LX, Zhou J, Sun HC. Serum vascular endothelial gro wth factor is a predictor of invasion and metastasis in hepatocellular carcinoma . *J Exp Clin Cancer Res* 1999,18:511-517
95 Niu Q, Tang ZY, Ma ZC, Qin LX, Zhang LH. Serum vascular endothelial growth factor is a potential biomarker of metastatic recurrence after curative res ection of hepatocellular carcinoma. *World J Gastroenterol* 2000;6: 565-568
96 Zhou J, Tang ZY, Fan J, Wu ZQ, Li XM, Liu YK, Liu F, Sun HC, Ye SL. Ex pression of platelet-derived endothelial cell growth factor and vascular endoth elial growth factor in hepatocellular carcinoma and portal vein tumor thrombus. *J Cancer Res Clin Oncol* 2000; 126:57-61
97 Tada K, Shiraishi S, Kamiryo T, Nakamura H, Hirano H, Kuratsu J, Kochi M, Saya H, Ushio Y. Analysis of loss of heterozygosity on chromosome 10 in pati ents with malignant astrocytic tumors: correlation with patient age and survival. *J Neurosurg* 2001;95:651-659
98 Bisgaard ML, Jager AC, Dalgaard P, Sondergaard JO, Rehfeld JF, Nielsen FC. Allelic loss of chromosome 2p21-16.3 is associated with reduced survival i n sporadic colorectal cancer. *Scand J Gastroenterol* 2001;36:405-409
99 Hirano A, Emi M, Tsuneizumi M, Utada Y, Yoshimoto M, Kasumi F, Akiyama F, Sakamoto G, Haga S, Kajiwara T, Nakamura Y. Allelic losses of loci at 3p25.1 , 8p22, 13q12, 17p13.3, and 22q13 correlate with postoperative recurrence in bre ast cancer. *Clin Cancer Res* 2001;7:876-882
100 Simoneau M, LaRue H, Aboulkassim TO, Meyer F, Moore L, Fradet Y. Chro mosome 9 deletions and recurrence of superficial bladder cancer: identification of four regions of prognostic interest. *Oncogene* 2000;19:6317-6323
101 Washburn JG, Wojno KJ, Dey J, Powell IJ, Macoska JA. 8pter-p23 dele tion is associated with racial differences in prostate cancer outcome. *Clin Cancer Res* 2000;6:4647-4652
102 Wong N, Lai P, Lee SW, Fan S, Pang E, Liew CT, Sheng Z, Lau JW, Johns on PJ. Assessment of genetic changes in hepatocellular carcinoma by comparative genomic hybridization analysis: relationship to disease stage, tumor size, and c irrhosis. *Am J Pathol* 1999;154: 37-43
103 Itano O, Ueda M, Kikuchi K, Shimazu M, Kitagawa Y, Aiura K, Kitajima M. A new predictive factor for hepatocellular carcinoma based on two-dimensiona l electrophoresis of genomic DNA. *Oncogene* 2000;19:1676-1683
104 Qin LX, Tang ZY, Sham JST, Ma ZC, Ye SL, Zhou XD. The associat ion of chromosome 8p deletion and tumor metastasis in human hepatocellular carci noma. *Cancer Res* 1999;59:5662-5665
105 Qin LX, Tang ZY, Ye SL, Liu YK, Ma ZC, Zhou XD, Wu ZQ, Lin ZY, Sun FX, Tian J, Guan XY, Pack SD, Zhuang ZP. Chromosome 8p deletion is associat ed with metastasis of human hepatocellular carcinoma when high and low metastati c models are compared. *J Cancer Res Clin Oncol* 2001;127:482-488
106 Zhang LH, Qin LX, Ma ZC, Ye SL, Liu YK, Ye QH, Wu X, Huang W, Tang ZY . Identification of allelic imbalances regions related to metastasis of hepatoce llular carcinoma: Comparison between matched primary and metastatic lesions in 2 2 patients by genome-wide microsatellite Analysis. *Int J Cancer* 2002 (submi tted)
107 Anker P, Stroun M. Circulating DNA in plasma or serum. *Medicina (B Aires)* 2000;60:699-702
108 Taback B, Fujiwara Y, Wang HJ, Foshag LJ, Morton DL, Hoon DS. Prognos tic significance of circulating microsatellite markers in the plasma of melanoma patients. *Cancer Res* 2001;61: 5723-5726
109 Nunes DN, Kowalski LP, Simpson AJ. Circulating tumor-derived DNA may permit the early diagnosis of head and neck squamous cell carcinomas. *Int J Cancer* 2001;92:214-219
110 Kennedy S. Proteomic profiling from human samples: the body fluid alt ernative. *Toxicol Lett* 2001;120:379-384
111 Tsai JF, Chuang LY, Jeng JE, Yang ML, Chang WY, Hsieh MY, Lin ZY, Tsa i JH. Clinical relevance of transforming growth factor-beta 1 in the urine of p atients with hepatocellular carcinoma. *Medicine (Baltimore)* 1997;76:213-226
112 King KL, Li AF, Chau GY, Chi CW, Wu CW, Huang CL, Lui WY. Prognostic significance of heat shock protein-27 expression in hepatocellular carcinoma an d its relation to histologic grading and survival. *Cancer* 2000;88:2464-2470
113 Osada T, Nagashima I, Tsuno NH, Kitayama J, Nagawa H. Prognostic sign ificance of glutamine synthetase expression in unifocal advanced hepatocellular carcinoma. *J Hepatol* 2000; 33:247-253
114 Kondo M, Yamamoto H, Nagano H, Okami J, Ito Y, Shimizu J, Eguchi H, M iyamoto A, Dono K, Umeshita K, Matsuura N, Wakasa K, Nakamori S, Sakon M, Monden M. Increased expression of COX-2 in nontumor liver tissue is associated with s horter disease-free survival in patients with hepatocellular carcinoma. *Clin Cancer Res* 1999;5:4005-4012
115 Chau GY, Wu CW, Lui WY, Chang TJ, Kao HL, Wu LH, King KL, Loon g CC, Hsia CY, Chi CW. Serum interleukin-10 but not interleukin-6 is related t o clinical outcome in patients with resectable hepatocellular carcinoma. *Ann Surg* 2000;231:552-558
116 Pirisi M, Toniutto P, Uzzau A, Fabris C, Avellini C, Scott C, Apollon io L, Beltrami CA, Bresadola F. Carriage of HFE mutations and outcome of surgica l resection for hepatocellular carcinoma in cirrhotic patients. *Cancer* 2000; 89:297-302
117 Yamamoto Y, Sakamoto M, Fujii G, Kanetaka K, Asaka M, Hirohashi S. Cl oning and characterization of a novel gene, DRH1, down-regulated in advanced hu man hepatocellular carcinoma. *Clin Cancer Res* 2001;7:297-303
118 Furumoto K, Arii S, Mori A, Furuyama H, Gorrin Rivas MJ, Nakao T, Iso be N, Murata T, Takahashi C, Noda M, Imamura M. RECK gene expression in hepatoce llular carcinoma: correlation with invasion-related clinicopathological factors and its clinical significance. Reverse-inducing-cysteine-rich protein with Kazal motifs. *Hepatology* 2001;33:189-195
119 Ye QH, Qin LX, Forgues M, He P, Kim JW, Peng AC, Simon R, Robles A, C hen YD, Ma ZC, Wu ZQ, Ye SL, Liu YK, Tang ZY, Wang XW. Gene expression profiling and supervised machine learning to define metastasis-related genes in human he patocellular carcinoma. *Nature Med* 2002 (submitted)

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