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• REVIEW •

Molecular profiling of hepatocellular carcinomas by cDNA microarray

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Abstract

Hepatocellular carcinoma (HCC) is one of the most common cancers in the world. Conventional diagnosis and treatment of this malignancy have been dismal and should be complemented by novel tools. The development and progress of HCC are believed to be caused by the accumulation of genetic changes resulting in altered expression of thousands of cancer-related genes, which can be measured by globe genetic analysis. Gene expression profiling of HCC has been employed to elucidate hepatocarcinogenesis and disclose molecular mechanisms underlying complex clinical features. Identifying phenotype-associated genes/profiles has impacts on current diagnosis and management strategy of HCC. In spite of some pitfalls of this technology and challenges in improving the research process, scrutinous validation of profiling data of HCC combined with other approaches will eventually benefit the patients.

Key words: Hepatocellular carcinomas; cDNA microarray; Gene expression profiling

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INTRODUCTION

Liver cancer is one of the most common causes of cancer deaths in the world[1,2]. Last decade statistical data show that HCC is the second major cause of cancer death in men and the third in women in Mainland China[3]. Viral hepatitis B (HBV) and C (HCV) and aflatoxin are major risk factors[4-6]. Chronic HBV infection has a strong association with hepatocellular cancer in China[7]. The early detection, treatment and prevention of this disease are disappointing and most patients are diagnosed in their late stages and management is unsatisfactory with high mortality and recurrent rates[3].

The successful clinical management of this human malignancy requires novel diagnostic and prognostic methods. At present, histopathologic evaluation of a tumor and tumor staging are the mainstays for guiding therapeutic interventions and predicting outcomes. However, the limitations of conventional methods are obvious. Tumors with identical histopathologies may progress differently, respond differently to therapy, and may be associated with different clinical prognosis, suggesting that additional parameters should be identified to predict disease outcomes[8,9]. Gene expression profiling of certain cancers may be able to serve as a complementary tool providing useful information[8].

Like most solid tumors, the development and progression of HCC are believed to be caused by the accumulation of genetic changes resulting in altered expression of cancer-related genes, such as oncogenes or tumor suppressor genes, as well as genes involved in different regulatory pathways, such as cell cycle control, apoptosis, adhesion and angiogenesis[6,10]. Because gene expression profiles provide a snapshot of cell functions and processes at the time of sample preparation, comprehensive analysis of the gene expression patterns of thousands of genes in certain tumor cells and comparison to the expression profile obtained from healthy cells and/or other cancer cells of different phenotype should provide insights into the consistent changes in gene expression that are associated with tumor cellular dysfunction and concomitant regulatory pathways. Current cDNA microarray technology enables investigators to measure the expression of thousands of mRNAs simultaneously in a biological specimen and therefore may provide comprehensive information for diagnosis and therapeutic interventions of tumors in the future[11].

Microarray technology now allows scientists and clinicians to identify qualitative and quantitative changes at RNA level in the development and progress of cancer[11-13]. In general, its HCC-oriented clinical applications can be categorized into three purposes: (1) to define the molecular profile of HCC distinct from non-cancerous liver and other tumor types to identify tumor-specific genes and liver-specific genes; (2) to describe gene expression profiles that correlate with clinical subsets, which will be helpful to disclose underneath mechanisms of HCC development and progress; and (3) to identify tumor-specific and clinical feature-related molecular markers, which will be helpful for cancer diagnosis, prediction of prognosis and response to treatment[8,14-16].

GENERAL GENE EXPRESSION PROFILE OF HCC

Numerous researchers have used microarray to profile gene expression pattern of HCC. Generally, the carcinogenesis and progression of HCC involve thousands of genes. Most of them are either "liver" specific or "cancer" specific. The genes characterizing HCC can mainly be categorized as those correlated with changes of liver function and differentiation, those correlated with dysregulation of signal pathways and those with tumor cell invasiveness.

Change of liver function and differentiation in HCC

HCC cells usually lose their normal function and differentiation. A large number of hepatocyte-specific gene products participating in the metabolism of nutrient factors and those responsible for the liver-synthesized proteins and detoxification enzymes are down-regulated[17-19]. Liver-synthesized functional proteins such as albumin, transferrin, and coagulation factors decreased. Enzymes implicated in biotransformation such as cytochrome P-450, metallothionein families and the glutathione S-transferases also decreased. HCC cells are distinguished for

increased glycolysis as seen in many cancers with up-regulated 6-phosphofructokinase-1[17]. All these alterations reflect de-differentiation of cancer cells.

In addition to the overexpression of alpha-fetoprotein (AFP), dozens of gene markers reminiscent of embryonization of hepatocytes are also noted[17].

Dysregulation of pathways in HCC and non-cancerous liver

Recent studies of cDNA microarrays suggest that heterogeneous hepatocarcinogenetic pathways exist[17-21]. These pathways associated with cell proliferation, cell cycle, apoptosis, and angiogenesis are dysregulated in carcinogenesis, such as p53 in cell cycle regulation, wingless (Wnt) signaling and MAPK pathways in signal transduction, cellular adhesion, and the TGF-beta/ insulin-like growth factor (IGF) axis.

In cell cycle control, negative regulators such as p27, p53, and p10 are less expressed, many cyclins and cyclin-dependent kinases are overexpressed. The combination of these events might drive the hepatocytes into cell proliferation[17]. Activation of Wnt signaling through mutations in beta-catenin (CTNNB1) contributes to the development of HCC and hepatoblastoma[22]. In the Wnt-β-catenin pathway, expressions of β-catenin and Wnt 2b were seen in some patients[17]. Aberrant expression of mitogen-activated protein kinase (MAPK) and associated proteins are also involved in the development of HCC[17,18,23]. The expression of insulin-like growth factor binding protein 3 (IGFBP3) was found to be down-regulated in HCC[18,21]. IGFBP-3 was reported to be a growth suppressor in various pathways. In the IGF receptor-dependent pathway, IGFBP3 mediates a wide variety of growth suppression signals such as TGF-β, retinoic acid, TNF-α and p53. Because retinoid is an accepted therapy to induce differentiation of cells in acute promyelocytic leukemia and is thought to help prevent development of HCC[24], reduced expression of this gene may play a crucial role in hepatocarcinogenesis. Apoptosis-related genes are reduced in HCC and in moderately poorly differentiated tumors, implying that a reduced rate of apoptosis is a major characteristic of tumor progression[17,18]. The abnormality of these pathways, therefore may represent a network required for the multistep process in HCC development.

In non-cirrhotic tissues, the down-regulation of a protein kinase C pathway inhibitor and the up-regulation of a PKC-regulated gene suggest activation of the PKC pathway[21].

Other genes changed in HCC

Other genes also show consistently elevated expression in HCC. These genes are implicated in a variety of cellular processes, including cell signaling, transcriptional regulation, RNA splicing, protein degradation, and cell adhesion[18,19]. Genes regulating the composition of the extra cellular matrix and the cytoskeleton such as fibronectin, tubulin alpha1, matrix metalloproteinase 14, osteonectin SPARC, Rho A are also found to be up-regulated[21]. These genes play important roles in cell motility and invasion.

GENE EXPRESSION PROFILES ASSOCIATED WITH CLINICAL FEATURES

Gene expression profiles generated by microarrays can be used to understand carcinogenesis and to outline clinical features among patients with histologically indistinguishable tumors[16]. Although considerable heterogeneity in gene expression profiles in HCC existed, a link could be seen between certain gene expression patterns and pathological features and some clinical features of HCC, including hepatitis virus status, clonal derivation, vascular invasion, and metastasis[19,21,25,26]. Analysis of gene expression patterns can be used as a classification tool to categorize cancer into various clinically relevant subgroups, which is currently impossible by other methods. These sub-categories often have distinct prognostic significances.

Expression patterns correlated with hepatitis virus status

Comparison of expression profiles between HBV-positive and HCV-positive HCCs implies that hepatitis viruses affect expression of dozens of genes in HCC in a type-specific manner, invoking partly different mechanisms of carcinogenesis. The genes differentially regulated in HBV- and HCV-positive HCCs are related to signal transduction, transcription, metastasis, and immune response[18,21,23].

Expression patterns show that the most differentially expressed genes are enzymes responsible for detoxification, which metabolizes carcinogens and/or anticancer agents[18,23]. One example is aldo-keto reductase family member AKR1C4, which encodes a key molecule for activating chemotherapeutic drugs or detoxifying xenobiotic carcinogens. Some genes exclusively overexpressed in HCV-positive HCCs are the phase I enzymes that convert several pro-carcinogens to activated metabolites. In contrast, expression of some genes of this cluster is preferentially repressed in HBV-positive HCCs while expression levels of the same genes are unchanged in most HCV-positive HCCs, which might reflect enhanced exposure of hepatocytes to activated carcinogens or radicals in HBV-related hepatocarcinogenesis[18].

Some exceptions convey important clinical meanings. Among detoxification related genes, GSTP1 is exceptionally up-regulated in HBV-positive HCC, although its mRNA level is relatively higher in both types of HCCs than in non-cancerous liver. Because GST conjugates reactive oxygen intermediates that are generated by many anticancer agents, the data suggest the efficacy of these anticancer agents in treating HCV-positive HCC and their limitations in treating HBV-positive HCC[18].

HBV- and HCV-positive HCCs exhibit involvement of different cellular pathways. TGF-β-induced encoding gene was found to be up-regulated in 44% of HCV-positive HCCs, suggesting the modulation of the TGF-beta pathway in HCV-positive HCC[21]. Imprinted genes IGF2 were found to be up-regulated specifically in HBV-positive HCC in comparison to both HCV-positive HCC and non-cancerous liver. IGF2 is known to be a mitogen often overproduced in tumors. This result suggests that up-regulation of the IGF-2 pathway may play an important role in the pathogenesis of HBV-positive HCC but not HCV-positive HCC[23].

In general, the distinct expression profiles of HBV- and HCV-positive HCCs suggest different hepatocarcinogenesis mechanisms and provide novel tools for diagnosis and treatment of HBV- and HCV-positive HCCs.

Expression patterns correlated with vascular invasion in HCC

Vascular invasion frequently occurs in HCC patients and seems to have an important role in tumor invasion and metastasis[27,28]. By microarray analysis, we identified genes correlated with the presence or absence of vascular invasion. Metalloproteinase 14 was found to be up-regulated on this list. The association of MMP14 expression with vascular invasion highlights the possible importance of MMPs in the progression of HCC and underscores their potential as therapeutic targets. Most of the genes that are expressed at lower levels in tumors with vascular invasion are "liver specific", consistent with the classical pathological observation that poorly differentiated HCC tumors tended to be more aggressive and invasive. One of the few genes in this group that does not belong to the liver-specific

cluster encode the metalloprotease ADAMTS1, which was recently shown to inhibit endothelial cell proliferation and to have antiangiogenic activity, warrants further investigation[19]. Genes associated with vascular invasion also involve small GTPase-related genes such as Rho C, Rho GAP8 and ARHGEF6, which are preferentially down-regulated in invasive tumors. Because the small-GTPase Rho family plays important roles in controlling cell motility and focal adhesions, alterations of their signaling pathways can enhance the migratory and invasive capacity of tumor cells and induce tumor invasion and metastasis.

Expression patterns correlated with immune response

Cancer-testis (CT) genes are expressed in a variety of human cancers, but not in normal tissues except for testis. These genes are recognized by cytotoxic T lymphocytes (CTLs) and represent promising targets for immunotherapy and gene therapy[29]. The most frequently expressed CT genes in HCC are SSX-1 and GAGE. Most HCC cases express at least one CT gene[30]. Okabe *et al*[18] found that expression of CT antigens such as MAGEC1, was repressed in poorly differentiated tumors. Reduced expression of genes encoding immune targets may confer a growth advantage by allowing tumor cells to escape from immune surveillance.

Clonal derivation of HCC elucidated by gene expression analysis

In HCC, multifocal growth may be due to intrahepatic metastatic spread or multicentric origin of clonal neoplasms[31]. This issue is of potential clinical and prognostic importance because the survival rate in the independent multicentric occurrence group was significantly better than that in the intrahepatic metastasis group[32]. These two kinds of nodules share similar microscopic features, and reliable differentiation could not be achieved using clinical or morphological criteria alone. Clonality determination using DNA fingerprinting with loss of heterozygosity (LOH) assay, comparative genomic hybridization (CGH), fluorescence *in situ* hybridization (FISH) and HBV integration pattern are reliable methods[9,33,34]

Microarray can also provide information on whether the tumor is multicentric in origin or single clonality followed by intrahepatic metastasis[35]. Analysis shows that all the tumor samples from each patient shared some similar gene expressions. Multiple clone-related tumor samples in the same patients could show different gene expression patterns due to divergent histories of mutations or chromosomal alterations. Overall, each independently arising tumor could be distinguished from other tumors of the same pathological type, whether they arise in the same patient or different patients, by a distinctive gene expression program that reflects the cell of origin and the unique sequence of genetic events[19,35].

Expression patterns correlated to HCC progress and metastasis

HCC associated with chronic liver disease evolves from pre-cancerous lesions and early HCC to advanced HCC. Nodule-in-nodule-type HCC (advanced HCC within early HCC) represents the transition from early to advanced HCC and, therefore, is useful in molecular genetic analysis of HCC progression during multistage carcinogenesis[36]. By microarray profiling, heat shock protein 70 (HSP70) has been identified as the most abundantly up-regulated gene in early HCC components. Further immunohistochemical examination of HSP70 revealed its significant overexpression in early HCC compared with pre-cancerous lesions and in advanced HCC compared with early HCC. Thus, HSP70 can be a sensitive marker of HCC progress, distinguishing early HCC from pre-cancerous lesion or non-cancerous liver[37].

HCC is one of the most aggressive human malignancies and its high mortality rate is mainly a result of intrahepatic metastases[3]. Ye *et al*[26] analyzed the expression profiles of HCC samples without or with intra-hepatic metastases by cDNA microarray-based gene expression profiling. Their results showed that the intra-hepatic metastatic lesions were indistinguishable from their primary tumors, regardless of tumor size, encapsulation and age of patients. But primary metastasis-free HCC was distinct from primary HCC with metastasis. These data indicate that changes favoring intra-hepatic metastasis are initiated in primary HCC.

By using a supervised machine-learning classification algorithm known as compound co-variate predictor (CCP), osteopontin (OPN) was identified with an average of 3-fold over-expression in HCC with thrombosis in the portal vein, but not in metastasis-free HCC. OPN is a secreted phosphoprotein highly expressed in patients with metastatic breast tumors and malignant lung, colon, and prostate tumors. An osteopontin-specific antibody can effectively block HCC cell invasion *in vitro* and inhibit pulmonary metastasis of HCC cells in nude mice[26]. Two recent papers also report OPN in HCC by differential display analysis[38,39] and one of them report that OPN mRNA overexpression is correlated closely with high-grade, late-stage, and early tumor recurrence, which lead to poorer prognosis and suggest OPN overexpression might serve as an unfavorable prognostic factor and a useful marker for predicting early recurrence in early-stage HCC[39]. Thus, osteopontin acts as both a diagnostic marker and a potential therapeutic target for metastatic HCC.

In recent reviews, breast-cancer metastasis suppressor 1 (BRMS1), KAI1, KISS1, NM23, E-cadherin, and TIMPs have been classified as metastasis suppressors, which by definition inhibit metastasis without blocking primary tumor growth[40,41]. In a study by Cheung *et al*[35], expression profiles of the primary nodules were compared with their corresponding intrahepatic metastatic nodules after determining clonality of all tumor nodules. A total of 90 clones were found to be correlated with intrahepatic metastasis[35]. Among these genes, BRMS1 has been shown to possess the functional capability of decreasing the metastatic potential of breast cancer cells[42] and warrants further investigation in liver cancer. Two other genes are CD53 and the EMP3, membrane proteins consisting of four transmembrane domains possess an important feature of KAI1, an accepted metastasis-related gene associated with lymph node or distant metastases in many cancer types[43,44]. These metastasis suppressors may provide novel therapeutic targets for the treatment of metastasis.

A direct way to identify metastasis-related genes is to compare gene expression profile of human HCC cell lines with different metastatic potentials. Based on gene expression profiles of MHCC97-L and HCCLM3, two HCC cell lines with similar genetic background but different in spontaneous metastatic potentials, were described by Li *et al*[45,46]. Twenty-five differentially expressed genes were found, including the decreased expression of cell cycle control genes Rb2, mismatch repair gene hMSH2, and PKC beta 2 and 7 and increased expression of MAPK kinase 6, *etc*[45,46]. These genes can be considered as potential markers to predict metastasis and targets for anti-metastasis intervention.

CLINICAL APPLICATION OF GENE EXPRESSION PROFILING IN HCC

Early detection biomarkers

The development of HCC is generally preceded by chronic

liver damage leading to cirrhosis. Screening liver cancer in patients at high risk by AFP and imaging diagnostics are conventional approaches for early detection. However, the cost effectiveness has long been debatable[47]. Half the HCC patients are AFP negative[48]. More appropriate molecular markers are needed to improve better early detection.

A major aim of the studies that profiled RNA expression patterns of human tumors is to identify gene products that may serve as useful biomarkers for cancer diagnosis[8]. Several research groups have focused on identifying subsets of genes that show differential expression between normal tissues or cell lines and their tumor counterparts to identify biomarkers. Xu *et al*[17] reported overexpression of dozens of gene markers reminiscent of embryonization or dedifferentiation of hepatocytes in addition to the overexpression of AFP in HCC. These genes include CD34, erythropoietin receptor, myeloid cell nuclear differentiation antigen (MNDA), early development regulator 2, and placental protein 15 (PP15), which are associated with hematopoiesis or embryonic development and hence are markers of fetal liver. Although most of these features may reflect the consequences of the cell transformation and thus are unlikely to play an essential role in carcinogenesis, some of them may be used as clinical diagnostic markers.

An alternative is to screen secreting molecules released by cancer. By sequence analysis for transmembrane domain or signal peptides, we were able to distinguish a subset of these genes whose products are likely to be either membrane-bound or secreted[19]. Diehn *et al*[49] developed a centrifugation-based methodology to separate nuclear and cytosolic mRNA transcripts encoding membrane-associated or secreted versus cytoplasmic proteins. These mRNA pools were then reversely transcribed, PCR amplified, sequenced, and spotted onto glass microarrays. The use of these methods may facilitate the identification of new biomarkers in the form of novel secreted proteins that may be detectable in serum or other body fluids. By raising antibodies against the products of these genes, scientists could identify new serum markers for detection and diagnosis of HCC. They may also facilitate the identification of new therapeutic targets in the form of membrane-bound tumor antigens that may help target chemotherapeutic agents to particular tumor tissues.

Differential diagnosis of liver-occupying lesions

Diagnosing liver-occupying tumors can be difficult in the setting of a poorly differentiated tumor or tumors with no known prior malignancy, especially when only fine needle aspiration biopsy is available. Differential diagnosis is of great importance when treatment is considered. Frequently, AFP and evidence of chronic hepatitis virus infection have been employed by clinicians to distinguish HCC from metastatic adenocarcinoma of neighboring gastrointestinal origin. However, these methods have their limitations. Microarray analysis can tell the origin of cancer cell[25,50].

Microarray analysis shows distinct gene expression profile of HCC from profiles of other metastatic lesions of extrahepatic origin. HCC samples and metastatic cancers can be clustered into two distinct groups, based on differences in their patterns of gene expression. The clustered liver-specific genes are consistently expressed at higher levels in HCC than in tumors of non-liver origin. Metastatic cancers originating from the same tissue are typically clustered together, expressing genes characteristic of the cell type origin. Thus, systematic comparison of the gene expression pattern in a metastatic tumor of uncertain origin observed in a large, diverse sample of tumors and normal tissues will allow reliable recognition of the primary tumor origin[19].

Predicting patients' survival

Recent advances in gene expression profiling technology have now made it feasible to use microarray technology in the routine management of cancer patients. Gene expression arrays have been demonstrated to be capable of predicting the therapy response, prognosis and survival of patients with various cancer types[16]. However, only few clinical applications of microarray in predicting HCC have been conducted.

Using a newly constructed predictive system consisting of 12 genes, Iizuka *et al*[51] found that the system correctly predicted early intrahepatic recurrence or non-recurrence with a positive predictive value of 88% and a negative predictive value of 95% in 27 cases within 1 year after curative surgery and suggested this system could serve as a new method for characterizing the metastatic potential of HCC.

In a gastric cancer project, we analyzed gene expression patterns in human gastric cancers and found PLA2G2A, a gene previously implicated as a modifier of the Apc (Min/+) (multiple intestinal neoplasia 1) mutant phenotype in mice, was of especially high variation in the expression level among tumors and significantly correlated with patient survival[52]. We are now attempting a similar study on HCC and hoping to find some new markers.

CHALLENGES

The potential of gene-expression profiling as a novel tool to improve diagnostic and prognostic prediction is very exciting. However, several challenges need to be addressed before routine clinical application. Some of these relate to the technology itself, others concern clinical utility.

The number of microarray-based studies identifying new genes or molecular pathways involved in tumor classification, cancer progression, or patient outcome is growing exponentially. There is a growing need for a standardization of the methodology, so that different datasets may be compared directly and meaningfully[13,53,54]. The technical challenges also include data interpretation. Two systems of databases, one containing expression data and the other containing annotation data are essential to mine the data for the research community[55].

In clinical aspect, major limiting factors for routine use of microarray in a clinical setting at present are cost and access to the microarray technology. It is likely that costs will decrease in the near future and that the technology will become increasingly user friendly and automated[11].

In sample preparation aspect, gene expression analysis using total RNA of bulk tissue usually cannot assign specific messages to particular cell types. Many microarray analyses including ours found distinct patterns of gene expression could provide molecular signatures of diverse cell types, including stromal cells, endothelial cells and infiltrating lymphocytes[19,21]. Cell-specific RNA expression profiling, may be crucial for a better understanding of the role of each distinct cell type within a physiological or pathophysiological setting. RNA profiling based on laser-controlled microdissection (LCM) of defined cells of a tissue now provides a useful tool for studying molecular crosstalk between different cell types within a tissue. LCM technique and suitable RNA amplification will potentially benefit the microarray studies[56].

Finally, validation of candidate genes obtained from genome-wide microarray analysis is critical[57]. Several approaches can be chosen, from basic Northern blotting or semiquantitative reverse transcription-PCR to *in situ* hybridization (ISH) using tissue microarrays. For most cases, immunohistochemistry (IHC) should be performed if antibodies are available, for only the protein is the ultimate functional form in cells. Two recent

examples described flowcharts validating candidate genes dysregulated in HCC[58,59]. Further more, gene expression profiling may need to be combined with other global approaches such as proteomics for optimization of its potential[53].

DISCUSSION

Gene expression profiles obtained by cDNA microarrays may help elucidate initiation, promotion, and progression of HCC. Identifying phenotype-associated genes/profiles has impacts on current diagnosis and management strategy of HCC. Despite pitfalls of technology and some existing challenges in research, microarray can provide reliable and productive profiling that cannot be easily reached with other methods. Clinicians are now approaching the era to initiate "bench to bedside" translation, during which the diagnostic, prognostic, and treatment response biomarker genes identified by microarray screening will be interrogated to provide personalized management of patients[11]. Coupled with more conventional biochemical analyses such as IHC and ELISA, microarrays will result in improvement of diagnostic and prognostic capability in cancer management.

In addition, gene expression profiling analysis also identifies cancer phenotype-associated genes represented by ESTs of unknown function. These genes may be useful as novel tumor markers and drug targets. Their functions warrant further investigations.

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REFERENCES

1 **Yu AS**, Keeffe EB. Management of hepatocellular carcinoma. *Rev Gastroenterol Disord* 2003; **3**: 8-24
2 **Yu MC**, Yuan JM, Govindarajan S, Ross RK. Epidemiology of hepatocellular carcinoma. *Can J Gastroenterol* 2000; **14**: 703-709
3 **Tang ZY**. Hepatocellular carcinoma-cause, treatment and metastasis. *World J Gastroenterol* 2001; **7**: 445-454
4 **Block TM**, Mehta AS, Fimmel CJ, Jordan R. Molecular viral oncology of hepatocellular carcinoma. *Oncogene* 2003; **22**: 5093-5107
5 **Stern MC**, Umbach DM, Yu MC, London SJ, Zhang ZQ, Taylor JA. Hepatitis B, aflatoxin B(1), and *p53* codon 249 mutation in hepatocellular carcinomas from Guangxi, People's Republic of China, and a meta-analysis of existing studies. *Cancer Epidemiol Biomarkers Prev* 2001; **10**: 617-625
6 **Thorgeirsson SS**, Grisham JW. Molecular pathogenesis of human hepatocellular carcinoma. *Nat Genet* 2002; **31**: 339-346
7 **Ding X**, Park YN, Taltavull TC, Thung SN, Jin X, Jin Y, Trung NS, Edamoto Y, Sata T, Abe K. Geographic characterization of hepatitis virus infections, genotyping of hepatitis B virus, and p53 mutation in hepatocellular carcinoma analyzed by *in situ* detection of viral genomes from carcinoma tissues: comparison among six different countries. *Jpn J Infect Dis* 2003; **56**: 12-18
8 **Macoska JA**. The progressing clinical utility of DNA microarrays. *CA Cancer J Clin* 2002; **52**: 50-59
9 **Wilkens L**, Bredt M, Flemming P, Mengel M, Becker T, Klempnauer J, Kreipe H. Comparative genomic hybridization (CGH) and fluorescence *in situ* hybridization (FISH) in the diagnosis of hepatocellular carcinoma. *J Hepatobiliary Pancreat Surg* 2002; **9**: 304-311
10 **Zhang LH**, Qin LX, Ma ZC, Ye SL, Liu YK, Ye QH, Wu X, Huang W, Tang ZY. Allelic imbalance regions on chromosomes 8p, 17p and 19p related to metastasis of hepatocellular carcinoma: comparison between matched primary and metastatic lesions in 22 patients by genome-wide microsatellite analysis. *J Cancer Res Clin Oncol* 2003; **129**: 279-286
11 **Macgregor PF**, Squire JA. Application of microarrays to the analysis of gene expression in cancer. *Clin Chem* 2002; **48**: 1170-1177
12 **Yasui W**, Tahara E. Gene diagnosis-introduction of new technology: the tenth international symposium of the Hiroshoma cancer seminar, October 2000. *Jpn J Cancer Res* 2001; **92**: 239-242
13 **Pusztai L**, Ayers M, Stec J, Hortobágyi GN. Clinical application of cDNA microarrays in oncology. *Oncologist* 2003; **8**: 252-258
14 **Mohr S**, Leikauf GD, Keith G, Rihn BH. Microarrays as cancer keys: an array of possibilities. *J Clin Oncol* 2002; **20**: 3165-3175
15 **Liu ET**. Classification of cancers by expression profiling. *Curr Opin Genet Dev* 2003; **13**: 97-103
16 **Yeatman TJ**. The future of clinical cancer management: one tumor, one chip. *Am Surg* 2003; **69**: 41-44
17 **Xu XR**, Huang J, Xu ZG, Qian BZ, Zhu ZD, Yan Q, Cai T, Zhang X, Xiao HS, Qu J, Liu F, Huang QH, Cheng ZH, Li NG, Du JJ, Hu W, Shen KT, Lu G, Fu G, Zhong M, Xu SH, Gu WY, Huang W, Zhao XT, Hu GX, Gu JR, Chen Z, Han ZG. Insight into hepatocellular carcinogenesis at transcriptome level by comparing gene expression profiles of hepatocellular carcinoma with those of corresponding noncancerous liver. *Proc Natl Acad Sci U S A* 2001; **98**: 15089-15094
18 **Okabe H**, Satoh S, Kato T, Kitahara O, Yanagawa R, Yamaoka Y, Tsunoda T, Furukawa Y, Nakamura Y. Genome-wide analysis of gene expression in human hepatocellular carcinomas using cDNA microarray: identification of genes involved in viral carcinogenesis and tumor progression. *Cancer Res* 2001; **61**: 2129-2137
19 **Chen X**, Cheung ST, So S, Fan ST, Barry C, Higgins J, Lai KM, Ji J, Dudoit S, Ng IO, Van De Rijn M, Botstein D, Brown PO. Gene expression patterns in human liver cancers. *Mol Biol Cell* 2002; **13**: 1929-1939
20 **Tannapfel A**, Wittekind C. Genes involved in hepatocellular carcinoma: deregulation in cell cycling and apoptosis. *Virchows Arch* 2002; **440**: 345-352
21 **Delpuech O**, Trabut JB, Carnot F, Feuillard J, Brechot C, Kremsdorf D. Identification, using cDNA macroarray analysis, of distinct gene expression profiles associated with pathological and virological features of hepatocellular carcinoma. *Oncogene* 2002; **21**: 2926-2937
22 **Taniguchi K**, Roberts LR, Aderca IN, Dong X, Qian C, Murphy LM, Nagorney DM, Burgart LJ, Roche PC, Smith DI, Ross JA, Liu W. Mutational spectrum of beta-catenin, AXIN1, and AXIN2 in hepatocellular carcinomas and hepatoblastomas. *Oncogene* 2002; **21**: 4863-4871
23 **Iizuka N**, Oka M, Yamada-Okabe H, Mori N, Tamesa T, Okada T, Takemoto N, Tangoku A, Hamada K, Nakayama H, Miyamoto T, Uchimura S, Hamamoto Y. Comparison of gene expression profiles between hepatitis B virus- and hepatitis C virus-infected hepatocellular carcinoma by oligonucleotide microarray data on the basis of a supervised learning method. *Cancer Res* 2002; **62**: 3939-3944
24 **Benoit GR**, Tong JH, Balajthy Z, Lanotte M. Exploring (novel) gene expression during retinoid-induced maturation and cell death of acute promyelocytic leukemia. *Semin Hematol* 2001; **38**: 71-85
25 **Su AI**, Welsh JB, Sapinoso LM, Kern SG, Dimitrov P, Lapp H, Schultz PG, Powell SM, Moskaluk CA, Frierson HF, Hampton GM. Molecular classification of human carcinomas by use of gene expression signatures. *Cancer Res* 2001; **61**: 7388-7393
26 **Ye QH**, Qin LX, Forgues M, He P, Kim JW, Peng AC, Simon R, Li Y, Robles AI, Chen Y, Ma ZC, Wu ZQ, Ye SL, Liu YK, Tang ZY, Wang XW. Predicting hepatitis B virus-positive metastatic hepatocellular carcinomas using gene expression profiling and supervised machine learning. *Nat Med* 2003; **9**: 416-423
27 **Chau GY**, Lui WY, Wu CW. Spectrum and significance of microscopic vascular invasion in hepatocellular carcinoma. *Surg Oncol Clin N Am* 2003; **12**: 25-34, viii
28 **Poon RT**, Fan ST. Evaluation of the new AJCC/UICC staging system for hepatocellular carcinoma after hepatic resection in Chinese patients. *Surg Oncol Clin N Am* 2003; **12**: 35-50, viii

29 **Bodey B**. Cancer-testis antigens: promising targets for antigen directed antineoplastic immunotherapy. *Expert Opin Biol Ther* 2002; **2**: 577-584
30 **Luo G**, Huang S, Xie X, Stockert E, Chen YT, Kubuschok B, Pfreundschuh M. Expression of cancer-testis genes in human hepatocellular carcinomas. *Cancer Immun* 2002; **2**: 11
31 **Nakashima O**, Kojiro M. Recurrence of hepatocellular carcinoma: multicentric occurrence or intrahepatic metastasis? A viewpoint in terms of pathology. *J Hepatobiliary Pancreat Surg* 2001; **8**: 404-409
32 **Shimada M**, Hamatsu T, Yamashita Y, Rikimaru T, Taguchi K, Utsunomiya T, Shirabe K, Sugimachi K. Characteristics of multicentric hepatocellular carcinomas: comparison with intrahepatic metastasis. *World J Surg* 2001; **25**: 991-995
33 **Wilkens L**, Bredt M, Flemming P, Klempnauer J, Heinrich Kreipe H. Differentiation of multicentric origin from intra-organ metastatic spread of hepatocellular carcinomas by comparative genomic hybridization. *J Pathol* 2000; **192**: 43-51
34 **Ng IO**, Guan XY, Poon RT, Fan ST, Lee JM. Determination of the molecular relationship between multiple tumour nodules in hepatocellular carcinoma differentiates multicentric origin from intrahepatic metastasis. *J Pathol* 2003; **199**: 345-353
35 **Cheung ST**, Chen X, Guan XY, Wong SY, Tai LS, Ng IO, So S, Fan ST. Identify metastasis-associated genes in hepatocellular carcinoma through clonality delineation for multinodular tumor. *Cancer Res* 2002; **62**: 4711-4721
36 **Saeki R**, Nagai H, Kaneko S, Unoura M, Yamanaka N, Okamoto E, Kobayashi K, Matsubara K. Intratumoral genomic heterogeneity in human hepatocellular carcinoma detected by restriction landmark genomic scanning. *J Hepatol* 2000; **33**: 99-105
37 **Chuma M**, Sakamoto M, Yamazaki K, Ohta T, Ohki M, Asaka M, Hirohashi S. Expression profiling in multistage hepatocarcinogenesis: identification of HSP70 as a molecular marker of early hepatocellular carcinoma. *Hepatology* 2003; **37**: 198-207
38 **Gotoh M**, Sakamoto M, Kanetaka K, Chuuma M, Hirohashi S. Overexpression of osteopontin in hepatocellular carcinoma. *Pathol Int* 2002; **52**: 19-24
39 **Pan HW**, Ou YH, Peng SY, Liu SH, Lai PL, Lee PH, Sheu JC, Chen CL, Hsu HC. Overexpression of osteopontin is associated with intrahepatic metastasis, early recurrence, and poorer prognosis of surgically resected hepatocellular carcinoma. *Cancer* 2003; **98**: 119-127
40 **Steeg PS**. Metastasis suppressors alter the signal transduction of cancer cells. *Nat Rev Cancer* 2003; **3**: 55-63
41 **Shevde LA**, Welch DR. Metastasis suppressor pathways—an evolving paradigm. *Cancer Lett* 2003; **198**: 1-20
42 **Meehan WJ**, Welch DR. Breast cancer metastasis suppressor 1: update. *Clin Exp Metastasis* 2003; **20**: 45-50
43 **Yunta M**, Lazo PA. Apoptosis protection and survival signal by the CD53 tetraspanin antigen. *Oncogene* 2003; **22**: 1219-1224
44 **Evtimova V**, Zeillinger R, Weidle UH. Identification of genes associated with the invasive status of human mammary carcinoma cell lines by transcriptional profiling. *Tumour Biol* 2003; **24**: 189-198
45 **Li Y**, Tang Y, Ye L, Liu B, Liu K, Chen J, Xue Q. Establishment of a hepatocellular carcinoma cell line with unique metastatic characteristics through *in vivo* selection and screening for metastasis-related genes through cDNA microarray. *J Cancer Res Clin Oncol* 2003; **129**: 43-51
46 **Li Y**, Tang Z, Ye S, Liu B, Liu Y, Chen J, Xue Q. Gene expression profile of human hepatocellular carcinoma cell lines with different metastatic potentials. *Zhonghua Zhongliu Zazhi* 2002; **24**: 533-536
47 **Yuen MF**, Lai CL. Screening for hepatocellular carcinoma: survival benefit and cost-effectiveness. *Ann Oncol* 2003; **14**: 1463-1467
48 **Johnson PJ**. The role of serum alpha-fetoprotein estimation in the diagnosis and management of hepatocellular carcinoma. *Clin Liver Dis* 2001; **5**: 145-159
49 **Diehn M**, Eisen MB, Botstein D, Brown PO. Large-scale identification of secreted and membrane-associated gene products using DNA microarrays. *Nat Genet* 2000; **25**: 58-62
50 **Ji J**, Chen X, Leung SY, Chi JT, Chu KM, Yuen ST, Li R, Chan AS, Li J, Dunphy N, So S. Comprehensive analysis of the gene expression profiles in human gastric cancer cell lines. *Oncogene* 2002; **21**: 6549-6556
51 **Iizuka N**, Oka M, Yamada-Okabe H, Nishida M, Maeda Y, Mori N, Takao T, Tamesa T, Tangoku A, Tabuchi H, Hamada K, Nakayama H, Ishitsuka H, Miyamoto T, Hirabayashi A, Uchimura S, Hamamoto Y. Oligonucleotide microarray for prediction of early intrahepatic recurrence of hepatocellular carcinoma after curative resection. *Lancet* 2003; **361**: 923-929
52 **Leung SY**, Chen X, Chu KM, Yuen ST, Mathy J, Ji J, Chan AS, Li R, Law S, Troyanskaya OG, Tu IP, Wong J, So S, Botstein D, Brown PO. Phospholipase A2 group IIA expression in gastric adenocarcinoma is associated with prolonged survival and less frequent metastasis. *Proc Natl Acad Sci U S A* 2002; **99**: 16203-16208
53 **Mariadason JM**, Augenlicht LH, Arango D. Microarray analysis in the clinical management of cancer. *Hematol Oncol Clin North Am* 2003; **17**: 377-387
54 **Kim JW**, Wang XW. Gene expression profiling of preneoplastic liver disease and liver cancer: a new era for improved early detection and treatment of these deadly diseases? *Carcinogenesis* 2003; **24**: 363-369
55 **Anderle P**, Duval M, Draghici S, Kuklin A, Littlejohn TG, Medrano JF, Vilanova D, Roberts MA. Gene expression databases and data mining. *Biotechniques* 2003; Suppl: 36-44
56 **Hergenhahn M**, Kenzelmann M, Grone HJ. Laser-controlled microdissection of tissues opens a window of new opportunities. *Pathol Res Pract* 2003; **199**: 419-423
57 **Chuaqui RF**, Bonner RF, Best CJ, Gillespie JW, Flaig MJ, Hewitt SM, Phillips JL, Krizman DB, Tangrea MA, Ahram M, Linehan WM, Knezevic V, Emmert-Buck MR. Post-analysis follow-up and validation of microarray experiments. *Nat Genet* 2002; **32** Suppl: 509-514
58 **Qiu W**, David D, Zhou B, Chu PG, Zhang B, Wu M, Xiao J, Han T, Zhu Z, Wang T, Liu X, Lopez R, Frankel P, Jong A, Yen Y. Down-regulation of growth arrest DNA damage-inducible gene 45beta expression is associated with human hepatocellular carcinoma. *Am J Pathol* 2003; **162**: 1961-1974
59 **Nakatsura T**, Yoshitake Y, Senju S, Monji M, Komori H, Motomura Y, Hosaka S, Beppu T, Ishiko T, Kamohara H, Ashihara H, Katagiri T, Furukawa Y, Fujiyama S, Ogawa M, Nakamura Y, Nishimura Y. Glypican-3, overexpressed specifically in human hepatocellular carcinoma, is a novel tumor marker. *Biochem Biophys Res Commun* 2003; **306**: 16-25

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