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Molecular prediction of early recurrence after resection of hepatocellular carcinoma

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ABSTRACT

The prognosis of hepatocellular carcinoma (HCC) remains poor. Vascular invasion, tumour multiplicity and large tumour size are the conventional poor prognostic indicators related to early recurrence. However, it is difficult to predict prognosis of each HCC in the absence of these indicators. The purpose of this study is to predict early recurrence of HCC after radical resection based on whole human gene expression profiling. Microarray analyses were performed in 139 HCC primary tumours. A total of 88 cases lacking the conventional poor prognostic indicators were analysed to establish a molecular prediction system characteristic for early recurrence in 42 training cases with two polarised prognoses, and to test its predictive performance in 46 independent cases (group C). Subsequently, this system was applied to another 51 independent cases with some poor prognostic indicators (group D). The molecular prediction system accurately differentiated HCC cases into poor and good prognoses in both the independent group C (disease-free survival [DFS]: $p = 0.029$, overall survival [OS]: $p = 0.0043$) and independent group D (DFS: $p = 0.0011$, OS, $p = 0.035$). Multivariate Cox regression analysis indicated that the clinical value of molecular prediction system was an independent prognostic factor ($p < 0.0001$, hazard ratio = 3.29). Gene expression pattern related to early intrahepatic recurrence inherited in the primary HCC tumour can be useful for the prediction of prognosis.

1. Introduction

Hepatocellular carcinoma (HCC) is a common malignancy worldwide and is currently the third major cause of cancer-related deaths in Japan.[1] Recent progress in diagnostic and treatment technologies has improved the long-term survival of patients with HCC, but the prognosis remains unfavourable. Surgical resection has been one of the mainstays in curative treatment of HCC. However, even after curative resection, 80% of patients develop intrahepatic recurrence and 50% die within 5 years.[2,3]

Some patients who have undergone curative resection suffer an unpredictable early fulminant recurrence in the remnant liver, and this is associated with dismal prognosis. Detection of cases with early recurrence at the time of resection is beneficial for better decision making for treatment. In this regard, a staging system for HCC according to clinicopathological findings has been applied to assess the risk of recurrence following resection.[4]

Vascular invasion, tumour multiplicity and large tumour size (tumours measuring more than 5 cm in diameter) are poor prognostic indicators of HCC,[2,5–7] and it is difficult to

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predict the prognosis of each case of HCC in the absence of these conventional indicators. However, the above-mentioned poor prognostic indicators are insufficient to predict the recurrence of HCC patients who undergo curative resection[21], thus new indicators are sought to help predict early intrahepatic recurrence developing after surgery in these patients.

Carcinogenesis is regulated by various changes on a genetic level, and several studies have discussed the phenomenon of cancer metastasis based on the analysis of various molecules. While it is useful to understand cancer progression, it is difficult to predict early recurrence with the analysis of a single molecule. The reason is that recurrence might be regulated by multiple molecular changes and interactions, and it might be difficult to explain the phenomenon of recurrence of HCC by a single molecule.[8–10] Therefore, it is important to conduct a comprehensive analysis of these molecules. The approach of microarray technology provides considerable information on cancer features and behaviour in individuals in several malignant tumours.[11–14] Several molecular and genetic studies have been reported on the progression of HCC and prediction of response of chemotherapy,[15–18] and some concluded that the specific gene expression patterns in HCC cancerous tissues could predict early intrahepatic recurrence.[19–22] However, it is still challenging to detect early recurrence tumours at the time of resection due to the complex pathogenesis of HCC. A recent study suggested that the strict selection of a homogeneous training set of patients in building the classifiers is essential to improve the predictability, reproducibility and validity of classifiers.[23]

In the present study, whole gene analysis was performed using a more clearly and strictly defined design set taking account of the complex pathogenic process of HCC, which reflected the prognosis more directly than previous reports with larger number of analyses.[24]

2. Materials and methods

2.1. Patients

A total of 139 HCC patients who had undergone hepatectomy at the Osaka University Hospital were enrolled in this study. All patients were followed up after resection for at least 3 months and the median follow-up time of survival cases in this study was 36 months (range, 12–87 months). Informed consent was obtained from all patients to use their surgical specimens and the clinicopathological data for research purposes. Histological classification was based on the Edmondson grading system and clinical stage was determined according to the Cancer of the Liver Italian Programme (CLIP). A mixture of RNA from the normal parts of liver specimens of seven patients with liver metastases from intestinal carcinomas was used as a reference for microarray analysis. None of the reference cases had hepatitis B or C (HBV or HCV, respectively) infection and their liver function tests were within normal values. All tissues were snap-frozen into liquid nitrogen and were stored at –80 °C.

2.2. Experimental design

Fig. 1 illustrates schematically our experimental design. Prediction of early recurrence in patients lacking the above-mentioned conventional poor prognostic indicators is clinically beneficial. In our study, we analysed patients lacking the aforementioned poor prognostic indicators to solve such a problem. To select the informative genes that are related to the phenomenon of early recurrence, we used two groups with polarised time course during the training phase. One group (group A) comprised cases with poor prognosis ($n = 21$), representing patients who developed multiple

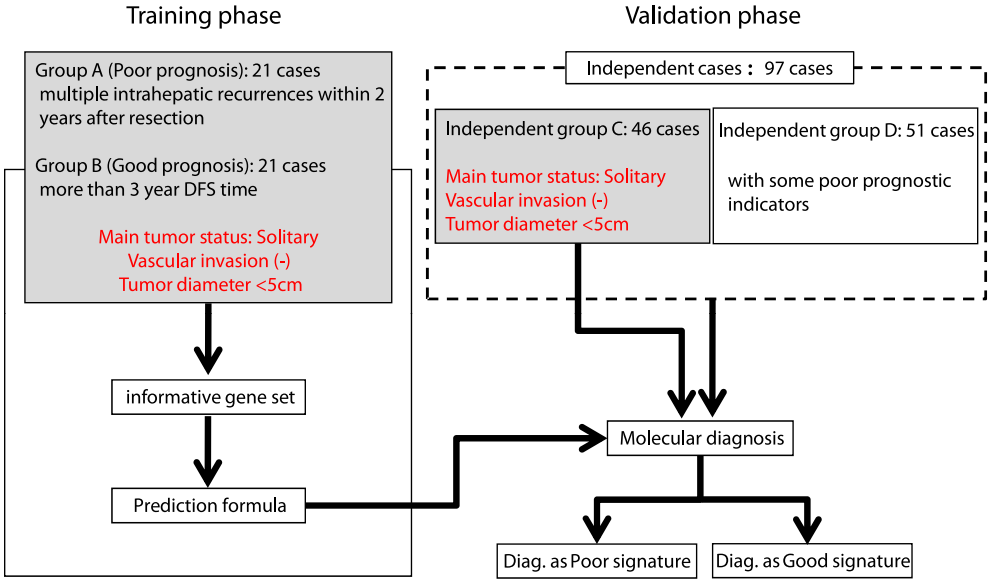

Fig. 1 – Schematic diagram of the experimental protocol. A molecular prediction system was constructed in the training phase. In the next step (validation phase), we applied this system to the independent group C ($n = 46$) and the entire group of independent cases ($n = 97$) comprising group C ($n = 46$) and group D ($n = 51$). Cases in grey coloured zones (Groups A, B and C) had similar clinicopathological conditions.

intrahepatic recurrences within 2 years after resection of the primary HCC. The second group (group B) comprised patients with satisfactory prognosis ($n = 21$), defined as more than 3-year disease-free survival (DFS) time. Table 1 summarises the clinicopathological features of patients of the two groups during the training phase. There were no differences between the two groups with regard to liver function tests and other clinicopathological variables except for the range of protein induced by vitamin K absence or antagonist II (PIVKA-II).

Based on the studies conducted in the training phase, a molecular prediction system was constructed using a set of informative genes. In the next step, we applied this system to another (independent) group C without any poor prognostic indicators as well ($n = 46$). The prediction system classified patients of group C into a 'poor signature' group (gene expression pattern resembled that of cases with poor prognosis) and a 'good signature' group (gene expression pattern resembled that of cases with good prognosis). Subsequently, we applied the prediction system to the independent group D ($n = 51$), which was composed of cases with positive status of some poor prognostic indicators. Finally, the independence of the diagnostic value of the molecular prediction results was verified by univariate and multivariate analyses using the whole independent cases, comprising patients of groups C and D.

2.3. *Microarray analysis*

Total RNA was extracted using TRIzol agent (Invitrogen, Carlsbad, CA), according to the instructions supplied by the manufacturer. Next, 2 μg of total RNA was used to synthesise double-strand cDNA that contained a promoter for T7 RNA polymerase. Amplified antisense RNA was synthesised by *in vitro* transcription of the cDNA templates by using the Amino Allyl MessageAmp aRNA kit (Ambion, Austin, TX). The reference and test sample were labelled with Cy3 and Cy5, mixed and hybridised on a microarray, AceGene Human oligo chip (DNA chip Research and Hitachi Software, Yokohama, Japan) DNA microarray. DNA microarray was used according to the instructions provided by the manufacturer (http://www.dna-chip.co.jp/thesis/AceGeneProtocol.pdf).

2.4. *Data analysis for postscanning*

The microarrays were scanned using ScanArray Lite and signal values were calculated using DNASIS array software (Hitachi Software Engineering Co., Yokohama, Japan). The local background was subtracted from each spot, and the ratio of the intensity of fluorescence from the Cy5 channel to the intensity of fluorescence from the Cy3 channel was calculated

Table 1 – Clinicopathological variables during the training phase.

	Poor prognosis group A ($n = 21$)	Good prognosis group B ($n = 21$)	*P* Value
Sex			
M	18	15	
F	3	6	0.452
Age, years			
<65	10	11	>0.999
⩾65	11	10	
HB infection			
+ve	8	11	0.535
–ve	13	10	
HC infection			
+ve	12	15	0.520
–ve	9	6	
Liver status			
Child A	19	15	0.239
Child B	2	6	
Tumour diameter, cm; mean (SD)	2.7 (0.8)	2.7 (1.1)	0.849*
AFP			
<400 ng/ml	18	18	>0.999
⩾400 ng/ml	3	3	
PIVKA-II			
<45 AU/ml	14	20	0.049
⩾45 AU/ml	7	1	
Capsule formation			
–ve	6	11	0.209
+ve	15	10	
Edmondson Grade			
I/II	13	16	0.504
III/IV	8	5	

P Values were calculated by the chi-square test, or by *Student *t*-test.

for each spot. Spots with intensity levels below the limit value were omitted. The ratio of expression level of each gene was converted to a logarithmic scale (base 2), and the data matrix was normalised to a median of 0 by standardising each sample.

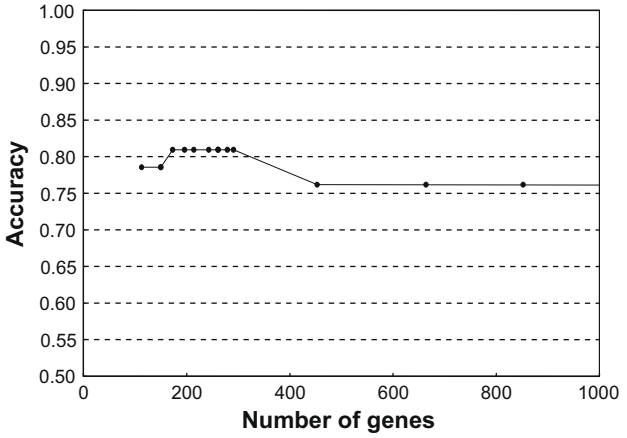

Fig. 2 – The accuracy curve based on weighted-voting algorithm with a leave-one-out cross validation. The accuracies in diagnosis of groups [ordinate] were plotted against the degree of *p*-value [abscissa]. The 172-gene set [P = 0.0004] marked the top accuracy. The accuracy was 80.2%. The P value was calculated by 10,000 times permutation test.

Genes with more than 15% missing data values in all samples in the training phase were excluded from the analysis. Missing data were compensated by averaging the expression data of 42 cases in the training phase.

2.5. *Construction of prediction system using gene expression patterns*

To detect the significant genes for prediction, we used permutation testing.[20] The original score of each gene (signal-to-noise ratio, $Si = (\mu A - \mu B)/(\sigma A + \sigma B)$, where μ and σ represent the mean and standard deviation of expression for each class, respectively) was calculated without permuting labels (responder or non-responder). The labels were randomly swapped and the values of S2N were calculated for the two groups. Repetition of this permutation 10,000 times provided a data matrix nearly the same as normal distribution. For each gene, the *P* value was calculated for the original S2N ratio with reference to the distribution of permuted data matrix. This model was evaluated by leave-one-out cross validation and the accuracy of each gene set was calculated based on the *P* value of the genes. As a supervised classification method, we adopted a weighted-voting (WV) algorithm.[13,14,19–22,25] We determined the optimal *P* value of the genes and classifier and constructed the prediction formula.

2.6. *Statistical analysis*

Clinicopathological indicators were compared using chi-square test and continuous variables were compared using

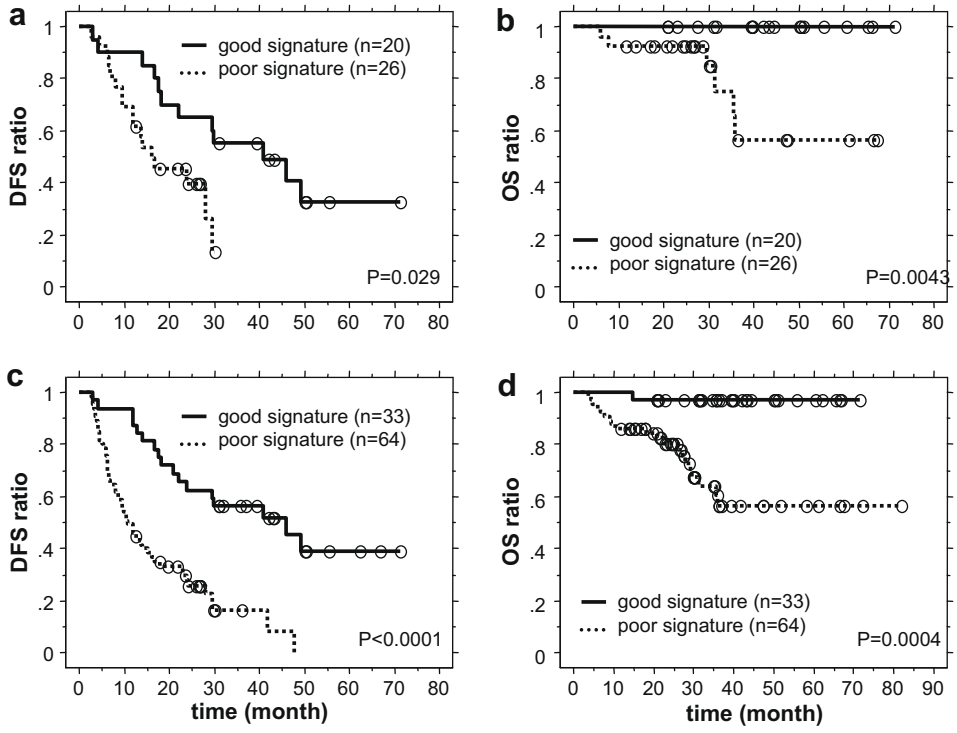

Fig. 3 – Disease-free survival curves and overall survival curves calculated using the Kaplan–Meier method for the independent cases. (a) DFS curves and (b) OS curves of the independent group C (*n* = 46). (c) DFS curves and (d) OS curves of the entire group of independent cases (*n* = 97) composed of groups C and D. Differences in survival curves were estimated by the log-rank test.

the Student *t*-test. Survival curves were computed using the Kaplan–Meier method, and differences between survival curves were compared using the log-rank test. To evaluate the risk associated with the prognostic variables, the Cox model with determination of the hazard ratio was applied; a 95% confidence interval was adopted. Statistical analyses were conducted using the SPSS software (version 11.0.1 J, SPSS Inc., Chicago, IL). We also performed network analysis using the Ingenuity Pathways Analysis (Ingenuity systems, Mountain View, CA; http://www.ingenuity.com), a web-based application.

Table 2 – Univariate analysis of the independent group C.

Parameter	Independent group C ($n = 46$)	*P* Value*	
		DFS	OS
Sex			
M	37	0.147	0.878
F	9		
Age, years			
<65	24	0.781	0.589
⩾65	22		
HB infection			
–ve	26	0.791	0.776
+ve	20		
HC infection			
–ve	18	0.467	0.980
+ve	28		
PIVKA-II			
<45 AU/ml	36	0.646	0.170
⩾45 AU/ml	10		
Capsule formation			
–ve	13	0.199	0.942
+ve	33		
Edmondson Grade			
I/II	27	0.479	0.479
III/IV	19		
CLIP score			
0–1	44	0.874	0.141
2–	2		
Liver status			
Child A	38	0.920	0.530
Child B	8		
AFP			
<400 ng/ml	38	0.724	0.374
⩾400 ng/ml	8		
Tumour diameter			
<5 cm	46	–	–
⩾5 cm	0		
Vascular invasion			
–ve	46	–	–
+ve	0		
Tumour multiplicity			
Single	46	–	–
Multiple	0		
Molecular-based diagnosis			
Poor prognosis	26	0.029	0.0043
Good prognosis	22		
Follow-up, months (median)	30 (4–81)		

* *P* Value was calculated by log-rank test according to the result of molecular diagnosis for DFS time.

Table 3 – Univariate analysis of the entire group of independent cases of independent groups C and D.

Parameter	Entire group ($n = 97$)	*P* Value*	
		DFS	OS
Sex			
M	80	0.604	0.582
F	17		
Age, years			
<65	42	0.892	0.850
⩾65	55		
HB infection			
–ve	51	0.584	0.416
+ve	46		
HC infection			
–ve	39	0.963	0.653
+ve	58		
PIVKA-II			
<45 AU/ml	73	0.897	0.387
⩾45 AU/ml	24		
Capsule formation			
–ve	19	0.730	0.748
+ve	78		
Edmondson grade			
I/II	49	0.015	0.169
III/IV	48		
CLIP score			
0–1	69	0.009	0.0024
2–	28		
Liver status			
Child A	81	0.229	0.032
Child B	16		
AFP			
<400 ng/ml	63	0.103	0.021
⩾400 ng/ml	34		
Tumour diameter			
<5 cm	72	0.062	0.021
⩾5 cm	25		
Vascular invasion			
–ve	82	0.187	0.0058
+ve	15		
Tumour multiplicity			
Single	65	0.0046	0.0033
Multiple	32		
Molecular-based diagnosis			
Poor prognosis	64	<0.0001	<0.0001
Good prognosis	33		
Follow-up, months (median)	30 (4–81)		

* *P* Value was calculated by log-rank test according to the result of molecular diagnosis for DFS time.

3. Results

3.1. Differentially regulated genes during the training phase

In the training phase, we examined the accuracy of prediction of HCC recurrence using full genes based on a WV algorithm with a leave-one-out cross validation approach. The accuracy of each gene set is shown in Fig. 2. The gene set of 0.0004% of *P* value using permutation test with 10,000 random trials marked the highest accuracy. We defined these differentially expressed 172 genes ($P = 0.0004\%$) as the informative gene set. Supplementary Table 1 provides a list of the informative genes. The results of molecular-based diagnosis system were correct in 34 of 42 cases. When compared with each annotated group, this system correctly classified 18 of 21 cases with poor prognosis and 16 of 21 cases with good prognosis in this set.

3.2. Results of molecular diagnosis of the independent group C

We adopted the prediction system constructed during the training phase to the independent group C, and compared DFS and overall survival (OS) of the patients between the two diagnosis groups (Fig. 3A). Both the DFS and OS ratios were significantly lower in patients diagnosed as 'poor signature'. The DFS curves showed significant difference between the two groups (log-rank test: $P = 0.029$) and all the seven patients who died of cancer were diagnosed as poor signature ($P = 0.0043$) (Fig. 3B). To compare other clinicopathological indicators with DFS and OS, we performed univariate analysis. Only molecular diagnosis was significantly different (Table 2).

3.3. Results of molecular diagnosis of independent group D

For cases of the independent group D, the DFS ratio and OS ratio were significantly lower in cases diagnosed as 'poor signature'. The log-rank test indicated that the DFS ratio ($P = 0.0011$) and OS ratio ($P = 0.035$) were significantly different between the 'poor signature' and 'good signature' groups (Figure not shown).

3.4. Results of whole independent cases and evaluation of prediction ability of molecular diagnosis relative to other conventional poor prognostic indicators

Our prediction system was further tested in the entire group of 97 cases (groups C and D). These cases were divided into 64 cases with poor signature and 33 cases with good signature based on the prediction system. Fig. 3C and D show the DFS and OS curves, respectively, for the two groups, according to the results of the prediction system. Kaplan–Meier survival estimates showed that DFS ratio was significantly lower in cases diagnosed as 'poor signature' than in patients diagnosed as 'good signature' ($P < 0.0001$). Twenty of 21 patients who died of cancer were of the 'poor signature' group and their OS curves were statistically different ($P = 0.0004$).

To compare our molecular prediction system with other conventional clinicopathological indicators, we performed univariate and multivariate analyses for DFS and OS. Univariate analysis of each factor for DFS time showed nearly significant differences with regard to Edmondson grade, AFP, tumour diameter, vascular invasion, tumour multiplicity and the result of molecular diagnosis (Table 3). To test the independence of the molecular prediction system, we performed multivariate Cox analysis. The result of the molecular prediction system was an independent factor ($P < 0.0001$), with a hazard ratio of 3.29 (95% CI 1.83–5.91) for the DFS ratio (Table 4). As for the OS ratio, the result of the molecular prediction system was also an independent factor ($P = 0.013$), with a hazard ratio of 13.28 (95% CI 1.72–102.63) (Table 4).

4. Discussion

The major finding of the present study was that early intrahepatic recurrence in patients who had undergone curative resection of HCC can be predicted accurately using our anal-

Table 4 – Results of multivariate analysis of the entire group of independent cases.

Variables	Hazard ratio	95% CI	*P* Value
Multivariate analysis of the entire group of independent cases (n = 97, DFS)			
Molecular diagnosis: poor signature (*versus* good signature)	3.29	1.83–5.91	<0.0001
Tumour multiplicity: multiple (*versus* single)	2.21	1.34–3.65	0.002
Edmondson grade: III/IV (*versus* I/II)	1.88	1.11–3.18	0.018
Tumour diameter: ⩾5 cm (*versus* <5 cm)	1.40	0.78–2.52	0.26
AFP: ⩾400 ng/ml (*versus* <400 ng/ml)	1.17	0.60–2.20	0.62
Vascular invasion: +ve (*versus* –ve)	0.93	0.46–1.87	0.84
Multivariate analysis of the entire group of independent cases (n = 97, OS)			
Molecular diagnosis: poor signature (*versus* good signature)	13.28	1.72–102.63	0.013
Tumour multiplicity: multiple (*versus* single)	3.06	1.16–8.06	0.024
Liver status: Child B (*versus* Child A)	2.38	0.90–6.29	0.08
Vascular invasion: +ve (*versus* –ve)	2.20	0.73–6.67	0.16
Tumour diameter: ⩾5 cm (*versus* <5 cm)	2.02	0.67–6.05	0.21
AFP: ⩾400 ng/ml (*versus* <400 ng/ml)	1.52	0.48–4.83	0.47
Edmondson grade: III/IV (*versus* I/II)	0.97	0.35–2.71	0.96

ysis system of gene expression patterns. Characteristic genes were selected by comparing the gene expression pattern between cases with multiple intrahepatic recurrences within 2 years and cases without recurrence over 3 years during the system training phase. The molecular prediction system accurately detected the high-risk group for early recurrence. Multivariate analysis identified molecular diagnosis, tumour multiplicity, and Edmondson grade as the independent factors. Taking into consideration that the majority of the patients who undergo curative resection become negative for the conventional poor prognostic indicators, molecular diagnosis could be potentially useful clinically for detecting patients at high-risk for early recurrence.

To improve the predictive accuracy, it is essential to clear the criteria of a homogeneous training set.[23] Our definition of the two groups was based on a study reported on the analysis of DFS ratio in HCC patients.[24] The DFS curve is composed of two regression lines. The majority of patients who developed recurrence within 2 years and who formed the first regression line were considered to have poor prognosis. On the other hand, the recurrence ratio of patients who showed no recurrence over a 3-year follow-up was almost the same as the annual relapse ratio of HCC in patients with hepatitis and their prognosis was better. This constant decrease in DFS ratio in the late recurrence cases is not usually observed in hepatectomised patients with liver metastasis from intestinal cancer.[26,27]

Recurrence of HCC is based on residual intrahepatic recurrence (IM) or multicentric metastasis (MC). IM is thought to originate from the primary cancer, while MC is considered to reflect a significant influence of the underlying liver status.[27,28] The two recurrence patterns are clinically important in patients with HCC where intrahepatic metastatic spread carries in general a poorer prognosis than that with multicentric nodules.[24] However, the conventional approach of histopathological examination is limited with regard to the differentiation of recurrence patterns as IM or MC.[29] With regard to the results of the validation phase, 17 patients survived for more than 3 years and only three of these 17 were diagnosed as poor signature and one of three cases was considered to have recurrence by metastasis from the primary tumour. On the other hand, 59 of 97 patients had intrahepatic recurrence within 2 years. This prediction system diagnosed these samples into 46 cases of poor signature and 13 cases of good signature. All 13 patients did not undergo a repeat resection, and thus pathological examination of recurrence pattern could not be conducted. However, as for the overall survival time in these 13 patients, only one died of cancer at 14 months postoperatively, while the remaining 12 patients remain alive for more than 21 months after surgery (range 21–48 months, median: 35 month). About half of the 13 patients had long survival though they had early recurrence. When we consider the relationship between study design and these results, the two groups diagnosed by our molecular-based diagnosis system may represent two recurrence patterns. The poor signature group may represent cases with recurrence due to IM, and the good signature group may represent cases with recurrence due to MC. This study may be clinically meaningful and helpful to solve the mechanism of recurrence patterns.

The prognoses of 42 patients during the training phase were polarised and those of the remaining 46 of the independent cases were intermediate. The 2-year survival ratio of the good signature independent cases was 65%, which was not as good as the annual relapse ratio of HCC. However, it is meaningful that the independent group C without any poor prognostic indicators could be divided into two groups of different prognoses. The reason for the discrepancy between the DFS ratio of cases diagnosed as good signature and annual relapse ratio is probably due to the fact that the independent group C did not include cases of extremely poor prognosis with early fulminant recurrence or cases of extremely good prognosis without long-term intrahepatic recurrence. Further analysis of cases with natural distribution of clinical status may help in moving the result of cases with good signature towards the annual relapse ratio.

In the conventional theory of metastasis, it is thought that tumours acquire the metastatic potential based on their progression and that metastasis occurs in the late phase. Based on this theory, recurrence could not be predicted by the analysis of the primary tumour. This theory was challenged recently by a new paradigm, which argues that the metastatic potential is not acquired in proportion to cancer progression but is already encoded in the primary tumour. Ramaswamy and colleagues[30] reported that a gene expression programme peculiar to metastasis may already be present in the bulk of some primary tumours and that a predictive diagnosis for metastasis was possible based on the analysis of the primary tumour profile. Several studies suggested that the molecular programme of primary tumour is generally retained in its metastasis.[31–33] Interestingly, Hoshida and colleagues[34] reported that the gene expression profiles in early-stage HCC tumours were highly associated with late recurrence (more than 2 years after resection) in the surrounding non-tumoural liver tissue but not in the tumoural tissue, indicating that environmental exposure leads to an increased potential of future malignant transformation. In this study, we evaluated the predictability of early recurrence using gene expression profiles of whole tumour tissue, based on the assumption that IM related to early recurrence might originate from the primary cancer.[27,28] For the entire group of independent cases, 78% of the recurrent cases within 2 years were diagnosed as poor signature. Some metastatic events may occur according to tumour progression, but cases with metastasis via the new paradigm should exist. Application of the theory of this paradigm may lead to the design of new diagnostic methods for cases in whom conventional clinicopathological parameters could not predict the prognosis.

Among the informative gene set, various genes correlate with cancer progression and carcinogenesis. PPARBP is regulated by RB18A and acts as a transcription cofactor by regulating the activity of p53wt transactivation on physiological promoters. Furthermore, downregulation of RB18A results in p53wt-dependent apoptosis.[35] RREB-1, a novel zinc finger protein, is involved in the differentiation response to Ras.[36] The Ras family is thought to be particularly important determinant of tumour initiation and progression.[37] BCL2 is one of the well-known tumour suppressor genes and is associated with recurrence and survival of HCC patients.[38,39] HDAC1 is reported to induce hyperacetylation of nucleosomal histones

in tumour cells, resulting in the expression of repressed genes that cause growth arrest, terminal differentiation and apoptosis.[40] The expression of HDAC1 is associated with prognosis of various carcinomas. BCOR is BCL6 co-receptor and is regulated by p53 and its characteristic expression is reported in various cancer cells.[41] This gene contributes to carcinogenesis in various malignancies such as B cell lymphoma and breast cancer.[42] BIRC5 is one of the major apoptosis regulators and is reported to be a prognostic marker of urothelial carcinomas and breast cancer.[43,44] These genes may serve as diagnostic markers for the development of HCC and help in the resolution of molecular mechanism of recurrence of HCC. To gain biological insights from these informative gene sets, we also used network analysis using Ingenuity Pathway Analysis. This analysis revealed that a few canonical signalling pathways, P38 MAPK signalling and PPARa/RXRa Activation signalling that are reported to be related to metastasis in human cancer,[45,46] harboured many of the upregulated informative genes (Supplementary Figure 1).

Our group has investigated the prediction of recurrence of various malignancies by gene expression profiling.[12,13,15,20,33,47] Kurokawa and colleagues[20] also reported a prediction model for HCC recurrence using a small-scale PCR-array system. They reported that early recurrence (within 2 year) in HCC patients could be predicted using 20-gene set after comparing cases with recurrence within 2 years and cases without recurrence over 2 years from 3072 primers.[20] In our study, cases with early intrahepatic recurrence within 2 years and reference cases without recurrence over 3 years during the training phase were defined based on DFS time of the characteristic recurrence patterns.[24] Furthermore, cases with common clinical background were analysed during the training phase using more strict criteria than previously reported using whole gene analysis, and accordingly, our results should be more reliable.

The report of Iizuka and colleagues[21] showed a correlation between gene expression, using a predictive system consisting of 12 genes, with early (within 1 year) post-hepatectomy intrahepatic recurrence, with a prediction accuracy of 89.3%. In their study, the DFS time of the reference group was more than one year and it is possible that characteristic recurrence patterns coexisted in the reference group. The study of Ho and colleagues[22] identified a molecular signature associated with vascular invasion (VI) in HCC and concluded that the signature could serve as a surrogate marker for predicting early recurrence after surgical resection.[22] Conventional prognostic indicators for early intrahepatic recurrence are not limited to vascular invasion only. A more direct approach should be considered for the prediction of early intrahepatic recurrence. While we did not analyse the reasons for the discrepancy in the prediction genes among the reported studies, we suspect that differences in clinical end-point may affect the results of the analysis and that accumulation of subtle differences in the dynamic range due to the platform of array might influence selection of the prediction genes.

In conclusion, the results of the present study showed that a characteristic gene expression pattern for early intrahepatic recurrence is encoded in primary HCC tumour and that gene profiling can be potentially helpful in predicting the prognosis of patients. Prediction of early recurrence of HCC may allow tailored treatment of individual patients and improvement of prognosis.

Conflicts of interest statement

None declared.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2008.12.019.

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