RESEARCH COMMUNICATION

Genetic Analysis of Liver Metastatic Cell Lines with Different Metastatic Potential

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Abstract

Metastasis is the major feature of malignant tumors that causes 90% of cancer deaths. Our laboratory has already established liver metastatic clones with YCC-16, isolated from the blood of a gastric cancer patient and expanded in vitro culture using a repeated orthotopic implantation method, and had reported biologic behaviour of the parental YCC-16, the orthotopic primary S1L0, and S1L1, S2L2 and S3L3 liver metastatic clones. Here, using these cell lines, we screened from chromosomal abnormalities using karyotype analysis and micro-CGH matching. There were 31 genes screened using PCA method which were functionally related to cell adhesion. Also, there were 23 genes selected which were related to the liver specific metastasis but excluded genes related to adhesion. There were 4 genes which demonstrated reduced or increased expression stepwise with passage. In conclusion, our results should contribute to exploring the mechanisms of liver metastasis by gastric cancer.

Keywords: Gastric cancer - orthotopic implantation - liver metastasis - genetic factors

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Introduction

The occurrence of metastasis in distant organs is the most major feature of malignant tumors and is the cause of 90% of cancer deaths (Douglas and Robert, 2000). The development of relevant in vivo models by orthotopic implantation has reappeared the metastatic process that is a more effective method for research of cancer metastasis. In 1990, Fidler indicated that implanting human tumor cells orthotopically into the corresponding organ of mude mice resulted in much higher metastatic rates (Fidler et al., 1990; Pettaway et al., 1996; Meng et al., 1999). Our lab had already established liver metastatic clones with YCC-16, which was isolated from the blood of a gastric cancer patient and was expanded in vitro culture using a repeated orthotopic implantation method in nude mice, providing a model for research the biologic behaviour and genetic change in the metastatic process.

Many labs (Fidler 1978; Reichner et al., 1996; Li et al., 2001; Shindo et al., 2001) had isolated and established subpopulations of tumor cell line with different metastatic potential by orthotopic implantation and suggested that the subpopulations had different biological characteristics such as tumor cell proliferation, migration, adhesion and invasion. However, comparison of differences among the subpopulations with different metastatic potentials in a tumor have been still rarely reported, particularly characteristics of the liver metastasis of gastric cancer on the biologic and genetic change.

These differences in biological and genetic characteristics include a variety of different properties such as tumor cell morphology; karyotype; loss and/or gain on the chromosome; gene expression profiling and proliferation, migration, and invasion patterns in vivo and in vitro. And some metastasis-related molecules have been understood, which included growth factor signaling molecules, chemokines, cell-cell adhesion molecules as well as extracellular proteases (Thomas and Meenhard, 2003). However, the genetic analyses so far performed to determine metastasis-related events was as yet insufficient to clearly explain the involved mechanisms (Byungsik et al., 2003).

In recent years, molecular cytogenetic studies such as CGH (Axel et al., 2001) have demonstrated their power in identifying recurrent chromosomal aberrations, and the cDNA microarray (Yoshitaka et al., 2002) analysis shows and allows the simultaneous expression analysis of thousands of genes from a test sample.

In this study, to exploring the mechanisms of liver metastasis of gastric cancer, based on liver metastatic clones with different metastatic potentials by orthotopic implantation of YCC-16 cell line that isolated from the blood of a gastric cancer patient, which were established by our lab, we investigated genetic characteristics of liver metastasis in gastric cancer using karyotype assay, micro-CGH and cDNA microarray.

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Materials and Methods

Cell culture

The parental YCC-16 cell line was from the blood of a gastric cancer patient in Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, Korea. The cells were maintained in the MEM (minimum essential medium, Invitrogen, CA, USA) media supplemented with 10% fetal bovine serum (FBS, Biofluids, MD, USA), streptomycin 100ug/ml and penicillin 100units (Invitrogen, CA, USA), in a humidified atmosphere of 95% air and 5% CO2 at 37°C. The culture media was changed three times weekly.

Orthotopic implantation and establishment of cell lines

Female athymic BALB/c nu/nu mice, 4-6 weeks old, weighing 18-22g were used for the experiment. Nude mice were obtained from Yonsei University College of Medicine, Division of Laboratory Animal Science. The mice were maintained in a laminar airflow cabinet under specific-pathogen-free conditions and were provided with sterile food, water and cages. The study protocol on mice was approved by the AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care International).

For the mouse inoculation, YCC-16 cells with log phase growth were harvested by trypsinization with trypsin-EDTA, washed with MEM and rewashed two times in phosphate buffered saline, and after cells were counted by hemocytometer, cells were resuspended in phosphate buffered saline. Using a 1ml tuberculin syringe fitted with a 30-gauge needle, YCC-16 cells ($1x10^6$/20ul/mouse) were implanted orthotopically under the serosal membrane in the greater curvature of the antrum in nude mice, we adopted a consecutive in vivo selection method as previously described (Kofi et al., 2001).

The mice were killed under deep anesthesia, when they showed signs of distress, the stomach, liver, regional lymph nodes, and other organs, resected to evaluate the metastatic potential of cell lines, and processed for routine histopathologic examination by haematoxylen-eosin (H&E) stain. The stomach tumor tissue and the liver with a few metastatic foci of YCC-16 cells were dissected aseptically and minced into small pieces with a scalpel in Hanks' balanced salt solution (HBSS, Invitrogen, CA, USA), and after centrifugation, the resulting material were resuspended in culture medium with 10% fetal bovine serum, streptomycin 100ug/ml and penicillin 100units plated onto cell culture flasks respectively. Several days later, two single cell suspensions were prepared by trypsinization and then cultured in vitro. The cells from the stomach tumor tissue were designated S1L0 and the cells from the liver metastatic foci were designated S1L1 respectively. The same procedure was repeated using S1L1 cells and the two liver metastatic clones were selected upon the two cycles of orthotopic implantation passage. Ultimately, four cell lines were established such as orthotopic primary tumor cell line S1L0 and the three liver metastatic clones which included S1L1, S2L2 and S3L3 (Figure 1). Each cell line was used for experiments at in vitro passage 5.

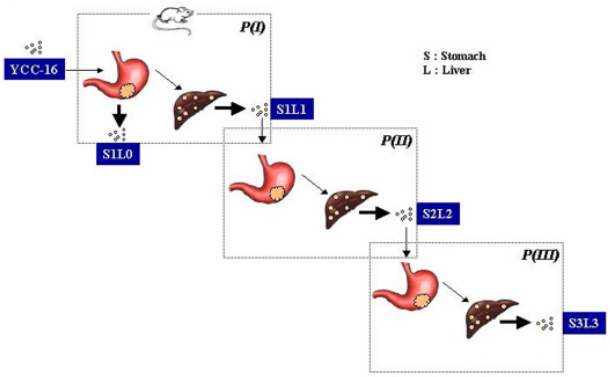

Figure 1. Schematic Flow. Selection and Establishment of the Orthotopic Primary Tumor Cell Line and the Metastatic Clones with Repeated Orthotopic Implantation Followed by Subsequent Passage

Karyotype analysis

Confluent cultures of five cell lines were treated with Colcemid (final concentration, 0.01mg/ml), washed with HBSS, and trypsinized. Single cell suspentions were centrifuged and the pellet was exposed to a hypotonic solution (0.06M KCl). After centrifugation, the cells were fixed in a mixture of methanol and acetic acid (3:1 by volume), washed twice with the fixative, and dropped on glass slides for air-drying preparations. The aged slides were trypsinized for 3.5min, rinsed in water and stained with Giemsa, rinsed again, air dried and examined under light microscope. 10 complete karyotypes for each cell line were prepared using the Genetiscan (PSI, Houston, Texas) for the possible identification of structural and numerical abnormalities. Chromosomal abnormalities which commonly presented in the five cell lines named by the letter "Mi" (common marker: M1, M2…Mi) and Chromosomal abnormalities which unique presented in each cell line of the five cell lines named by the letter "mi" (unique marker: m1, m2…mi).

Total RNA and genomic DNA isolation

Total RNA from the five cell lines was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) and the genomic DNA of each cell line was isolated using phenol/ chloroform/ isoamylalcohol. The quantity of the total RNA and genomic DNA were determined by a spectrophotometer, GeneSpec III (Hitachi, Tokyo, Japan), and the quality was assessed by agarose gel electrophoresis and an assay on a Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA).

Micro-CGH

The micro-CGH used a human cDNA chip (CMRC-GT, Seoul, Korea) containing 17, 000 spots. Reference XX placenta genomic DNA (6ug) and the test samples, the genomic DNA (6ug) of each cell line of the five cell lines were digested at 37°C, for 2h by DpnII (NEB, Beverly, USA) and cleaned up by QIAquick PCR purification kit (QIAgen, Dusselolord, Germany) according to the manufacturer's instructions. The test samples of the five cell lines were labeled with Cy5 and were individually co-hybridized with the Cy3-labeled XX placenta DNA using a Bioprime labeling kit (Invitrogen, CA, USA). Unincorporated nucleotides were removed by using PCR purification Kit. Eluted probes were then mixed

and supplemented with 30ug of Human Cot-1 DNA (Gibco-BRL, Gaithersburg, MD, USA), 20ug of poly-A RNA (Sigma, Saint Louis, Missouri, USA), 100ug of yeast t-RNA (Gibco-BRL, Gaithersburg, MD, USA) and 288ul of 1M TE buffer (PH 8.0). This probe mixture was concentrated using a Microcon-30 (Millipore, Bedford, MA, USA). For the final probe preparation, 15.3ul of 20x SSC (PH 8.0) and 2.7ul of 10% SDS were added to the probe mixture to make a total volume of 90ul. The probe was denatured by heating for 2min at 100°C and then applied to the 17k human cDNA chip. Microarray was hybridized at 65°C for 16h in hybridization chamber (GenomicTree Co., Daejun, Korea) with maintenance of humidity by 3.5x SSC. After the hybridization, the slides were washed for 2-5min in 2x SSC with 0.1% SDS, followed by 1x SSC with 0.1% SDS, 0.2x SSC, and then rinsed twice with 0.05x SSC. After washing, slides were spun at 600 rpm for 5min.

cDNA microarray

The cDNA microarray used a human cDNA chip (CMRC-GT, Seoul, Korea) containing 17,000 spots. The test sample, total RNA (50ug) of the each cell line of the five cell lines, were labeled with Cy5 and were individually co-hybridized with the Cy3-labeled. Labeling was performed at 42°C for 2h and the slides were hybridized in hybridization chambers (GenomicTree Co., Daejun, Korea) at 65°C for 16h. After the hybridization, the slides were washed for 2-5min in 2x SSC with 0.1% SDS, followed by 1x SSC with 0.1% SDS, 0.2x SSC, and then rinsed twice with 0.05x SSC. After washing, slides were spun at 600 rpm for 5min. The experiments repeated three times.

Image scanning and analysis of cDNA microarray and micro-CGH

Slides were scanned with GenePix 4000B scanner (Axon Instruments, Foster Cicy, CA, USA) and TIFF images were analyzed with GenePix 4.1 software (Axon Instruments, Foster Cicy, CA, USA). Foreground and background intensities of both Cy3 and Cy5 were calculated for each spot and exported into GenePix Array List (GAL) files. To correct the differences caused by labeling efficiencies, pin-tip Lowess normalization was applied which subtracted the median intensity ratio of Iog2 (R/G) from the Iog2-transformed data and GeneSpring software (SiliconGenetics, USA) was used for cDNA microarray analysis. According to the relationship between five cell lines, the unsupervised hierarchical clustering was performed and the gene expression profiling was observed. The genes were selected, which were expressed differentially in five cell lines, using a Principle Component Analysis (PCA), and the supervised hierarchical clustering of there selected genes was performed. The annotation of the selected genes was performed using the Stanford Online Universal Resource for Clones and Expressed sequence tags (SOURCE) (http://source.stanford.edu/cgi-bin/source/sourceSearch).

The CGH analyzer and map viewer using S-Plus (CAMVS) program that had been made from our lab, was used for micro-CGH analysis, and ratio of Iog2 (R/G) greater than 0.68 was defaulted for chromosomal gain, and ratio of Iog2 (R/G) less than -0.68 was defaulted for chromosomal loss (Axel et al., 2001).

Statistical analysis

Comparison of genetic change between two cell lines were analyzed using the two-sample Mann-Whitney test by STATA 8.0 statistical software package (StataCorp, Texas, USA) and p value of < 0.05 was considered statistically significant.

Results

Establishment of liver metastatic clones

We had already established liver metastatic clones with different metastatic potentials by orthotopic implantation of YCC-16 cell line that was isolated from the blood of a gastric cancer patient in our lab. As shown in Table 1, in the passage II occurred liver metastasis only in 2 of 5 (40%) mice, in the passage III and passageIV, also occurred liver metastasis only in 3 of 5 (60%) mice respectively.

Genetic change on the chromosome

The modal chromosome number of the five cell lines were follows such as YCC-16: 57, S1L0: 55, S1L1: 54, S2L2: 55 and S3L3: 55 respectively. Four chromosomal abnormalities the commonly presented in the five cell lines named by M1, M2, M3 and M4. Tentative identification of the common marker was:

M1:[(del(1) (q ter), del(1) (p ter) and der(1) t(1;9) (9q→1p ter)]

M2: [der(9) t(9;7) (7p→9p ter) and der(9) t(9;?) (?→9p ter)]

M3: [del(12) (12p ter)]

M4: [der(16) t(16;7) (7q→16q ter)]

The tetrasomy 1 appeared in all the cell lines and the trisomy 2, 5, 12, 15 and 20 also appeared all the five cell lines.

Four chromosomal abnormalities the unique presented in each cell line of the five cell lines named by m1, m2, m3 and m4. Tentative identification of the unique marker was:

m1: der (2) t(2;?) (?→2q ter)

m2: del(3) (3q ter)

m3: del(11) (11p ter)

Table 1. Incidence of Distant Metastasis During Different Passages by Orthotopic Implantation

Passage	Number of mice	Liver Metastasis Incidence (%)	Lung Metastasis Incidence (%)	Spleen Metastasis Incidence (%)	Lymph node Metastasis Incidence (%)
I	3	3 (100)	3 (100)	3 (100)	3 (100)
II	5	2 (40)	0 (0)	0 (0)	0 (0)
III	5	3 (60)	0 (0)	0 (0)	0 (0)
IV	5	3 (60)	0 (0)	0 (0)	0 (0)

PassageI, PassageII, PassageIII and PassageIV stand for YCC-16, S1L1, S2L2 and S3L3, respectively

Table 2. Selection of Genes with Loss and/or Gain on the Chromosome by Matching Karyotype to Micro-CGH

Chromosome number	Karyotype	Micro-CGH Gene ID	Locus
1	M1,tetrasomy	AA774608	1q32.1
		R00859	1q21
		T51539	1p36.13
		R07296	1q25
		AI289196	1p13
		AA453293	1p31
		AA446557	1p34.2
		AA191692	1p35.3
		AI421774	1p36.33
		AI668789	1p36.33
2	m1, trisomy	AI290868	2q36
		R43483	2q31.1
		AI471796	2q13
		AA936776	2q13
5	Trisomy	AA418564	5q13.1
		A482508	5q13.1
		AI262957	5q12.2-q13.3
		AA281797	5q12.2-q13.3
		AA283007	5q11-q12
7	(S1L0, S1L1, S3L3)monosomy	AW004731	7p12
		AA453459	7p14
		N65969	7p15
		AA973492	7q21-q22
9	m2	AI565346	9p13.3-p12
11	m3	AA427924	11p15.2
12	M3, trisomy	N49725	12q14.3-q15
		AA504489	12q14.1
		H20659	12q11-q12
		AI356535	12p11.2-p11.1
15	Trisomy	AA250966	15q22.1
		AI352369	15q15.1
16	M4	AI337344	16q23.1
17	m4, (YCC-16) trisomy	H14372	17q24.3
		AA481504	17q24.3
		AA911712	17q24.2
		R37145	17q22-q23
		H59204	17q21.3
20	Trisomy	AI986098	20q13.33
		AA931822	20q11.23
		AA931820	20q11.21

m4: der (17) t(17;?) (?→17q ter)

Marker m4 was presented in YCC-16, m1 and m2 both were presented in S1L0, m1 was presented in S1L1, m3 was presented in S2L2, m2 and m3 both were presented in S3L3. The monosomy 7 appeared in S1L0 and S1L1, the trisomy 3 appreared in S1L0 and S3L3 (Table 2). Above results denoted that the five cell lines with similar genetic background but the five cell lines with variant genetic change on chromosome.

The results of micro-CGH analysis that was consistent with the location of unbalanced structural chromosomal rearrangements identified by karyotype analysis. Gene gain and/or loss on the chromosome 1-3, 5, 7, 9, 11, 12, 14-17, 20 and xx chromosome could be observed by micro-CGH (Figure 2), and detailed genetic change on the chromosomes was as follows, such as YCC-16

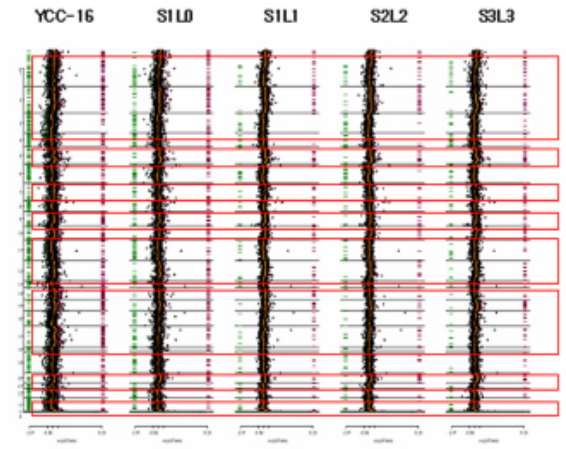

Figure 2. Chromosomal Gains and Losses in The Five Cell Lines. X axis represented cell line and Y axis represented chromosome number. Gains were shown as red lines on the right of each scatter plot and losses as green lines on the left. Each spot represented one gene in scatter plot. Chromosomal losses and gains observed on chromosome 1-3, 5, 7, 9, 11, 12, 14-17, 20 and XX (as shown with red boxes)

showed gain and/or loss of 746 genes, 622 genes for S0L1, 158 genes for S1L1, 183 genes for S2L2 and 185 genes for S3L3 respectively. Analysis of matched CGH and karyotype data demonstrated that the 40 genes were selected from on chromosome 1-2, chromosome 4-5, chromosome 7-12, chromosome 15, chromosome 17 and chromosome 20, and they included these commonly changed and variously changed genes (Table 2).

Hierarchical clustering

To investigate the relationship of gene expression profiling among the five cell lines, unsupervised hierarchical clustering with 15,723 genes of variant expression was performed using a cDNA microarray and identified difference of gene expression patterns among the parental YCC-16, the orthotopic primary S1L0 and the liver metastatic clones. The five cell lines based on similarity in their gene expression patterns were preferentially clustered into three groups, such as YCC-16 group, S1L0 group and liver metastatic clones group in the first, and furthermore were grouped two groups in the second, such as YCC-16 group with S1L0 group and liver metastatic clones group, whereas liver metastatic clones group was classified into two groups such as S1L1, S2L2 group and S3L3 group that showed difference of gene expression among liver metastatic clones (Figure 3).

Genetic changes on chromosome matched to gene expression

From the 40 genes which were selected from karyotype data matching to CGH data to investigate the genes expression level, only 10 genes presented genetic change on the expression level but 30 genes did not among the 40 genes (Table 3). Within the 10 genes, macrophage stimulating, pseudogene 9, that were related to the cell proliferation, presented chromosomal loss and appeared to increased expression in the gene expression level, while the other genes appeared reduced or increased expressions

Table 3. Result of the Genes with Gain and/or Loss on Chromosomes Matched to their Expression Levels

name	Symbol	Gene ID	Locus	YCC16	S1L0	S1L1	S2L2	S3L3
				Expression (fold change)				
Macrophage stimulating, pseudogene 9 MSTP9	T51539	1p36.13	1.45	2.29	2.01	2.05	2.07	
EST		AA453293	1p31	2.97	2.15	1.83	1.73	2.19
General transcription factor IIH, polypeptide 2, 44kDa	GTF2H2	AI262957	5q12.2-q13.3	1.92	2.73	2.78	3.04	3
General transcription factor IIH, polypeptide 2, 44kDa	GTF2H2	AA281797	5q12.2-q13.3	2.02	2.32	2.36	2.51	2.23
PFTAIRE protein kinase 1	PFTK1	AA973492	7q21-q22	#VALUE!	-2.29	-2.1	-2.26	-2.24
Tumor protein p53 inducible nuclear protein 1	TP53INP1	AA459364	8q22	-1.42	-1.67	-1.95	-1.88	-1.84
Spondin 1, extracellular matrix protein	SPON1	AA427924	11p15.2	-2.16	-2.44	-2.25	-2.16	-2.44
Contactin 1	CNTN1	H20658	12q11-q12	2.54	3.26	2.66	2.71	3.52
Bicaudal D homolog 1 (Drosophila)	BICD1	AI356535	12p11.2-p11.1	1.17	1.08	1.65	1.71	1.24
CDC6 cell division cycle 6 homolog (S. cerevisiae)	CDC6	H59204	17q21.3	1.56	0.89	1.56	1.72	1.31

Shown are the gene name, symbol, chromosomal location and expression level results of the gene from the 17k microarray (CMRC-GT chip) expression of YCC-16, S0L1, S1L1, S2L2 and S3L3

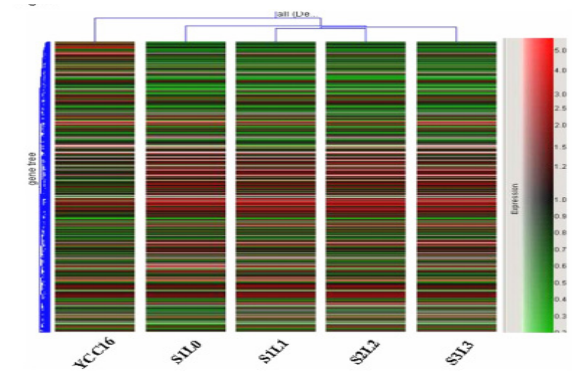

Figure 3. Hierarchical Clustering of the Variation Patterns with 15,723 Genes in the Five Cell Lines. The dendrogram at the top of the figure represented the hierarchical clustering of the cell lines based on similarity in their pattern of expression of these genes. Each gene was represented by a single row of colored boxes; each cell line was represented by a single column. In the color scale, red squares represented log expression ratios > 0 (overexpressed in the tumor cell), whereas green squares represented log expression ratios < 0 (underexpressed in the tumor cell). The color scale of saturation was proportional to the magnitude of the log expression ratio from the median value, with red indicating the greatest overexpression and green indicating the greatest underexpression. Black squares represented log expression ratios of 0 (similar expression in both cancer cell and reference), whereas gray squares indicated insufficient data. Five cell lines well clustered into three groups, such as YCC-16 group, S1L0 group and S1L1, S2L2, S3L3 group, whereas S1L1, S2L2, S3L3 group was separated furthermore into two groups such as S1L1, S2L2 group and S3L3 group

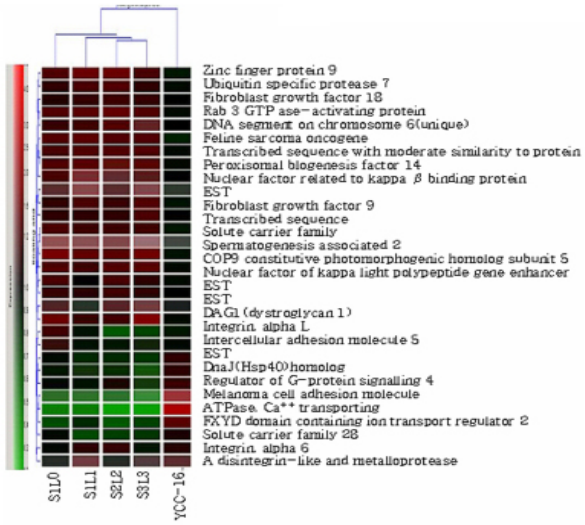

Figure 4. Hierarchical Clustering of the Five Cell Lines with 31 Genes Which were Related to the Cell Adhesion. The genes were selected by PCA method

in expression level, following the chromosomal gene loss or gain. 3 genes including PFTAIRE protein kinase 1, tumor protein p53 inducible nuclear protein 1 and spondin 1, presented to reduced expression but the other 7 genes appeared to increased expression among the 10 genes. Macrophage stimulating, pseudogene 9 and general transcription factor IIH, polypeptide 2, 44kDa appeared to reduced expression in YCC-16 than that of the other cell lines.

Selection of adhesion related genes

The 31 genes were screened by PCA method, which were functionally related to the cell adhesion and were differentially expressed in the five cell lines. They included EST unknown genes and zinc finger protein 9, fibroblast growth factor 18, platelet-derived growth factor receptor-like, solute carrier family, integrin, alpha L, integrin alpha 6, and dystroglycan (DAG1) etc, which were known for associating with the cell adhesion (Figure 4).

Selection of liver metastasis related genes

The 23 genes were screened, which were maybe functionally related to the liver specific metastasis but excluded adhesion related genes by comparison of YCC-16 and the liver metstatic clones. The 23 genes included EphA4 that was associated with signal transduction and CXCL1 that was known as one of chemokines (Figure 5).

Selection of genes changed stepwise with passages in liver metastatic clones

The 4 genes were screened by PCA method, which appeared to reduced or increased expression stepwise with subsequent passages in liver metastatic clones. They included R38343 that was unknown genes and transmembrane protein 16A, RAC/CDC42 exchange

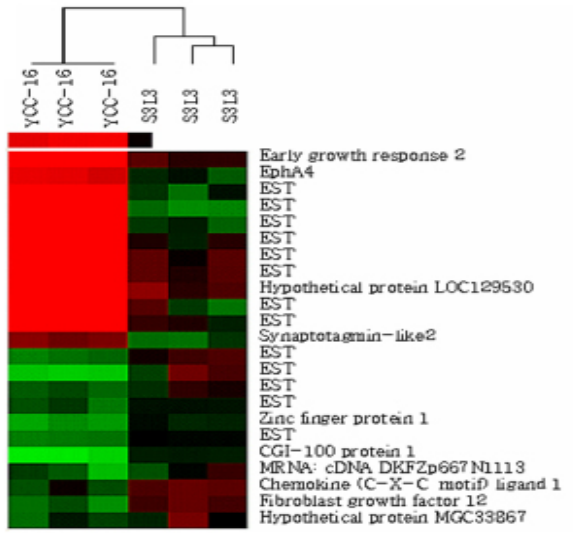

Figure 5. Hierarchical Clustering of the Five Cell Lines with 23 Genes Which were Related to the Liver Metastasis. The genes were selected by PCA method

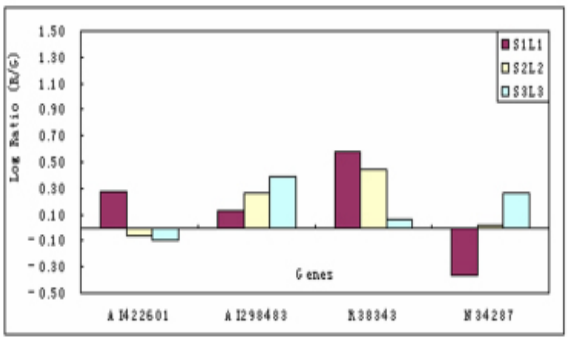

Figure 6. Selection of the Genes Changed Stepwisely with Passages in Liver Metastatic Clones. N34287: UNC-5 homolog C; AI298483: RAC/CDC42 exchange factor; AI422601: Transmembrane protein 16A; R38343: EST

factor, and UNC-5 homolog C, which were known as associated with signal transduction and cell proliferation (Figure 6).

Discussion

Gastric cancer cell lines have been studied, showing that certain genetic change might be associated with particular biological behavioral changes (Hippo et al., 2001; Nomura et al., 2001; Mori et al., 2002; Sakakura et al., 2002). Karyotype analysis indicated that YCC-16 and the other cell lines came from same clonal origin but had different properties, which might promote the five cell lines with biological divergence. Within the 10 genes, which were screened from the chromosomal abnormalities matching to the gene expression levels, macrophage stimulating, pseudogene 9 presented chromosomal loss but presented increased expression, and it might be that the genomic DNA has not been fragmentated fully and/or included intron after fragmentation that causes interaction with cDNA on the chip. In addition, poor labeling causes experimental mismatch too (Wei et al., 2001). Jane Bayani et al (Jane et al., 2002) proposed that correlating expression analysis (by cDNA microarray) with chromosomal dosage change or rearrangement (by karyotype and micro-CGH) would allow better identification of key genetic changes in cancer. The 10 genes were located at thechromosome 1, 5, 7, 8, 11, 12 and 17, and most genes were involved in cell proliferation, differentiation and signal transduction pathway. Chromosomal aberration frequently occurred on the chromosome 1, 5, 7, 11 and 17 in gastric cancer (Kuniyasu et al., 1994; ElRifai et al., 1998; SangWook et al., 1998; Aron et al., 1999; Sakakura et al., 1999). Macrophage stimulating, pseudogene 9 and general transcription factor IIH, polypeptide 2, 44 kDa appeared to reduced expression in YCC-16 than that of the other cell lines. The 3 genes showed chromosomal gene losses and reduced expression, which might be associated with the tumor suppression, and in contrast, the 7 genes showed chromosomal gene gains and increased expression, which might be correlated with the tumor development and progression (Wei et al., 2001). Among the 3 genes, tumor protein p53 inducible nuclear protein1 (TP53INP1) may regulate p53-dependent apoptosis through phosphorylation of p53 (Shu et al., 2001), and spondin 1 was related to regulating matrix organization, cell-cell interactions and cell guidance that was associated with tumor growth (Richard and Tucker, 2004).

Various molecules such as adhesion molecules, cytokines, and chemokines play important roles in preferential metastasis (Anja et al., 2001; Mossaad et al., 2001; Ann et al., 2002). Our animal models showed that YCC-16 as a metastatic precursor, caused multiple organ metastasis, whereas the liver metastatic clones caused the liver specificity of metastasis. The 23 genes were selected from YCC-16 and the liver metstatic clones, which might be functionally related to the liver specific metastasis but excluded the adhesion related genes. The chemokine CXCL1 was overexpressed in liver metastatic clones and CXCL4, a superfamily of CXCL1, was associated with bone metastasis in breast cancer (Yibin et al., 2003). Further research would be needed on whether CXCL1 overexpression is related to liver metastasis.

The liver metastatic clones such as S1L1, S2L2 and S3L3, which were from orthotopically implanted tumor models through 3 passages, appeared with different genetic change using karyotype assay, micro-CGH and cDNA microarray. The 4 genes were screened by using a PCA method in the liver metastatic clones which appeared to reduced or increased expression stepwise with subsequent passages, because they have regularity expression change, therefore might be considered associated with liver metastasis. Among the 4 genes, RAC/CDC42 which appeared to increased expression stepwise with passages in liver metastatic clones. RAC/CDC42 is a family of Rho GTPase, modulate cell-cell adhesion by regulating cadherin activity (Filippo and Erkki, 1999) and the gene as a signaling molecule with bi-directional crosstalk, by "outside-in" pathway regulates cell proliferation, survival and motility and by "inside-out" pathway regulates expression and function of cell surface molecules (Dwayne and David, 2002; John and David, 2002). Our genetic study showed that signal transduction molecules, adhesion molecules and chemokines were involved in liver metastasis of gastric cancer.

In summary, based on liver metastatic clones with YCC-16 established before in our lab, which isolated from blood of a gastric cancer patient and expanded in vitro culture by using a repeated orthotopic implantation method, we investigated genetic change with the five cell lines such as parental YCC-16, orthotopic primary S1L0 and the liver metastatic clones including S1L1, S2L2 and S3L3. The difference of genetic change between YCC-16 and liver metastatic clones occurred in differential expression of adhesion and chemokine molecules and the difference of genetic change between the liver metastatic clones appeared in differential expression of the molecules which were involved in cell proliferation and signal transduction. Although further functional analysis is needed, our results can lay the foundation for exploring the mechanisms of gastric cancer liver metastasis.

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