Validation of Angiogenesis DNA Chip with Drug-Treated Human Fibrosarcoma Cell

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Abstract

DNA microarray is a powerful tool that provides the massive information of gene expression profiles. Angiogenesis is an important pathway known to be related with several pathologies, including cancer. We developed DNA chip containing angiogenesis-related genes so that researchers could study angiogenesis based on changes of gene expression. For fabricating angiogenesis DNA chip, we selected 150 angiogenesis-related genes from databases, amplified the selected cDNA from 10 human cDNA libraries using PCR, and arrayed in duplicate on a slide glass. In order to validate the functionality of the angiogenesis DNA chip, we examined gene expression changes in HT1080 cells treated with either basic fibroblast growth factor and fetal bovine serum, well known pro-angiogenic factors, or trichostatin A, a known angiogenesis inhibitor, using the DNA chip. The obtained microarray data showed that each pro- and anti-angiogenic factor had an opposing effect on the gene expression changes in HT1080 cells, which are consistent with previously reported data. These results demonstrate that the angiogenesis DNA chip can be a useful tool towards angiogenesis-related researches.

Purpose of Experiment

Angiogenesis is an important pathway known to be related with rheumatoid arthritis, psoriasis, diabetic retinopathy, artery stiffening and inflammation reaction, as well as cancer. Despite the importance of angiogenesis, researchers have found difficulty to study on the genes related with angiogenesis, because DNA chips focusing for angiogenesis have not been developed and commercialized yet.

Here we report the development of an angiogenesis DNA chip using PCR products of about 150 angiogenesis-related genes, which consists of identical two blocks. Expression profiles of angiogenesis DNA chip with human HT1080 fibrosarcoma cells treated with angiogenesis inhibitor/activator were investigated and validated the usefulness of angiogenesis DNA chip for angiogenesis related researches.

Results

For fabricating angiogenesis DNA chip, we selected 150 angiogenesis-related genes (Table 1), and PCR-amplified the cDNA fragments from 10 human cDNA libraries. The DNA fragments confirmed by sequencing were spotted onto a slide glass surface-coated with amino residue in a regular array (Fig. 1).

In order to compare the change of the expression profile by angiogenesis inhibitor/activator, HT1080 human fibrosarcoma cells were treated with bFGF, 10% FBS, or 10% FBS containing trichostatin A (TSA) for 12 hrs. After treatment, total cellular RNA was extracted from each sample and labeled by Cy3 or Cy5. The labeling products were hybridized with the angiogenesis DNA chip at 65℃ for 16 hrs. After washing the DNA chip, the fluorescence image were obtained and analyzed to get the data (Fig. 2). Validation of duplicate microarrays was achieved through both scan image (Fig. 3) and signature correlation analysis (Fig. 4). Finally, the gene expression information showed that TSA has an adverse effect to FBS, and the genes activated by TSA were depressed by FBS (Fig. 5), consistent with previous reported results.

Materials and methods

PCR of angiogenesis-related genes

PCR primers for amplification of 150 angiogenesis-related genes were designed as following; 1) poly (A) up-stream and Alu sequence were eliminated, 2) sequence homology of PCR target regions was minimized, 3) amplicons were 0.2 ~ 1 Kb in size. DNA fragments were amplified from 10 human cDNA Libraries, validated by sequencing, and prepared over 7.2 ug per fragment.

Fabrication of angiogenesis DNA chip

DNA fragments were spotted duplicate in two blocks by 417 arrayer (Affymetix) and post-processed by Schena, M. et al. (1995). Following post-processing, QCs were performed through 3 steps; non-specific hybridization QC, position hybridization QC, and surface QC. QC-passed angiogenesis DNA chips were stored in a dark desiccator and used for further analysis.

Expression profiling of HT1080 cell

Human HT1080 fibrosarcoma cells were treated by bFGF or 10% FBS as angiogenesis activators, and by TSA in 10% serum as angiogenesis inhibitor for 12hrs following serum starvation for 12 hrs. HT1080 cells were harvested and RNAs were extracted. RNA from treated cells were labeled with Cy5-dUTP by reverse-transcription (test) and RNA from serum-starved cells with Cy3-dUTP (control). Labeled products were set onto the angiogenesis DNA chip and incubated at 65℃ for 16 hrs in a dark water-bath. Following wash, scanning by 428 scanner (Affymetrix), raw data extraction, and statistical analysis using GeneSight data analysis software ver. 3.5 (BioDiscovery) were achieved.

Micorarray data analysis condition

- Duplicate experiment
- Average signal intensity for analysis
- Eliminate spots in intensity of lower than 100
- Choose spots in all intensities of higher than 100 (intersection)
- LOWESS normalization
- Select genes of expression ratio with 2-fold up and/or down
- Compare expression profiles to the results of previous articles.

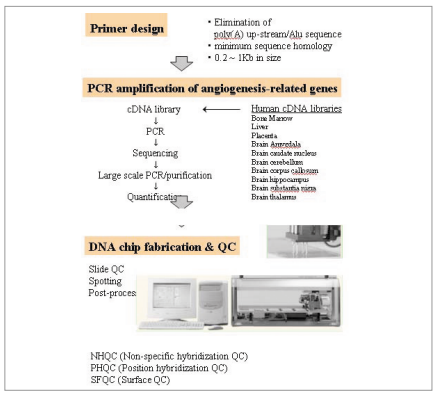

Fig 1. Fabrication of Angiogenesis DNA chip

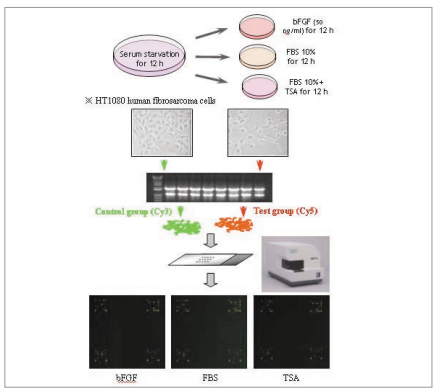

Fig 2. Experimental conditions and procedures. FBS 10% was used as an angiogenic activator and histone deacetylase (HDAC) inhibitor, TSA was used as an angiogenesis inhibitor. The microarray scanning images were from previous version of angiogenesis chip.

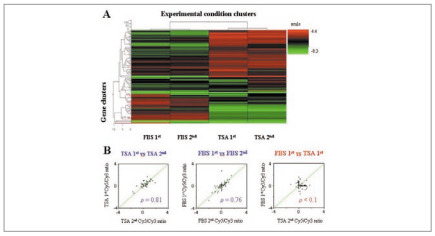

Fig 3. DNA microarray image analysis. Duplicate microarray images from each experimental condition (FBS and TSA) are shown. Each scanning image of duplicates is well correlated.

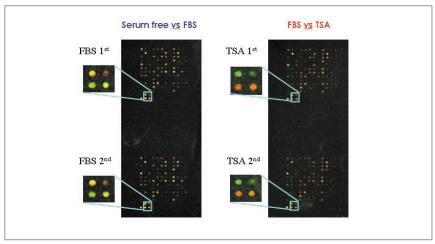

Fig 4. Signature correlation analysis of each duplicate microarray data. (A) Hierarchical clustering analysis showed that each duplicate data (FBS 1st vs FBS 2nd and TSA 1st vs TSA 2nd) is well correlated. (B) Signature correlation coefficient values (ρ) also demonstrate that each duplicate data is well correlated. In contrast, signatures between FBS and TSA are significantly different from each other (ρ < 0.1).

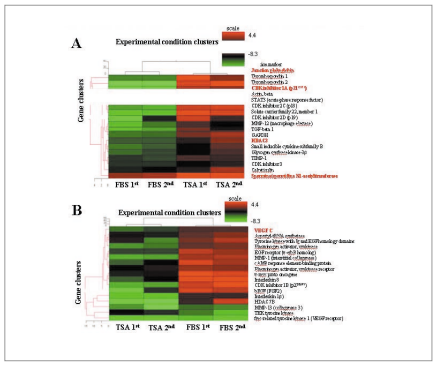

Fig 5. Microarray data validation using gene expression information. Up-regulated genes (A) and down-regulated genes (B) in response to TSA are shown. Red colored gene names were previously reported as HDAC target genes. Note that TSA and FBS have opposing effects on gene expressional regulation each other.

Conclusions

- 150 angiogenesis-related genes were selected
- Angiogenesis DNA chip was manufactured using sequence-verified PCR fragments
- Gene expression changes in HT1080 cells were examined using Angiogenesis DNA chip
- Angiogenesis DNA chip developed in this study is expected to be highly effective for the research on angiogenesis-related diseases and drug development.

Referenses

1. Eisen, M.B., Spellman, P.T., Brown, P.O. and Botstein, D. 1998. Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci.* USA **95**:14863.
2. Schena, M., Shalon, D., Davis, R.W. and Brown, P.O. 1995. Quantitative monitoring of gene expression patterns with complementary DNA microarray. *Science* **270**:467.
3. Kim JH, Shim JS, Lee SK, Kim KW, Rha SY, Chung HC, Kwon HJ. 2002. Microarray-based analysis of anti-angiogenic activity of demethoxycurcumin on human umbilical vein endothelial cells: crucial involvement of the down-regulation of matrix metalloproteinase. *Jpn J Cancer Res.* **93**(12):1378.

Discrimination of High Quality Japonica Rice Cultivars by High-Throughput Genotyping with MegaBACE1000

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Introduction

Grain quality is a major target on rice breeding in Korea as well as many other rice producing countries of the world. The advent and utilization of molecular markers has facilitated the understanding of the genetic similarity and complex quantitative traits. Molecular markers are able to produce high polymorphic alleles in rice and are suitable for evaluating genetic diversity among closely related rice cultivars including japonica rice(Ji et al. 1998, Kwon et al. 1999). Simple sequence repeats (SSRs) and other DNA sequence-tagged site markers can be genotyped more rapidly and useful to evaluate the diversity of Korean japonica rice cultivars having high-eating quality.

Objectives

The objective of this study was to develop a systematic and flexible method for assembling simple sequence repeats (SSRs) marker with capillary electrophoresis(CE) and a laser detection system for high throughput discrimination of high quality japonica rice cultivars.

Materials and Methods

● Materials

- Fifty seven Korean high quality japonica rice cultivars were provided by National Institute of Crop Science (NICS).

● Methods

- DNA extraction

Genomic DNA was extracted from fresh cultivars according to the modified procedure as described by Causse *et al.* (1994).

- PCR

o Selected 6 microsatellite markers (FAM-labeled)
o *Perfect* PreMix v2.0 (TaKaRa code R512.)

- SSR Fragment Analysis

o MegaBACE 1000 (Amersham Biosciences, USA)
o Analysis Software - Genetic Profiler ver1.1
o Size Standard - MegaBACE ET550-R

Results and Discussion

● PCR reactions were optimized for the proper amplification of each microsatellite marker which are previously selected for rice genotyping at NICS and estimated on the MegaBACE1000 with a size standard labeled with ET-ROX.

● The size variance of each allele was less than 0.5bp in replicated trials. Figure 2 shows the analysis of "Kwang-An Byeo" with 6 microsatellite markers.

● Fifty-seven high quality japonica rice cultivars were classified into 6~12 groups based on the fragment polymorphisms of each alleles (Table 1), and discriminated each rice cultivar as a unique identification code (Table 2).

● By using an automatic analysis system, the speed and the accuracy of fingerprinting? analysis for japonica rice cultivars were increased much better than those of traditional PAGE gel analysis system.

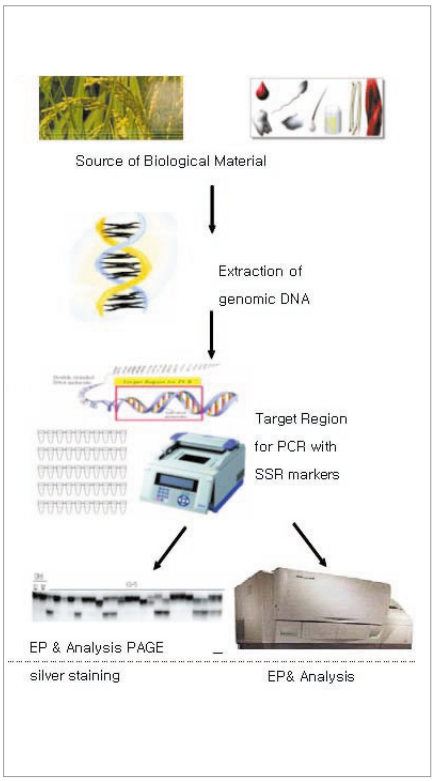

Fig. 1. Overall procedure of rice genotyping

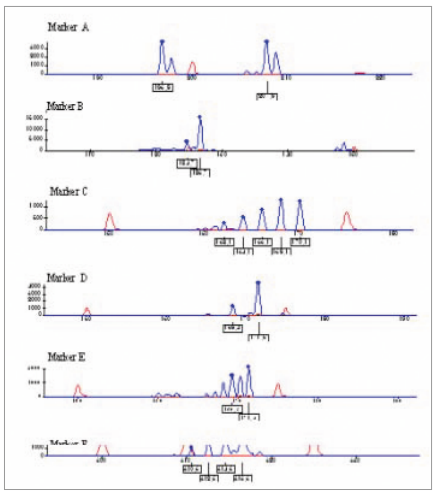

Fig. 2. Electropherogram of "Kwang-An Byeo" by using six-microsatellite markers.

A marker		B marker		C marker		D marker		E marker		F marker	
Fragment size	code	Fragment size	code	Fragment size	code	Fragment size	code	Fragment size	code	Fragment size	code
208	1	122.8	1	156	1	185.9	1	160.6	1	262.7	1
212.3	2	126.7	2	158.1	2	168.3	2	169.4	2	302.5	2
214.1	3	134.6	3	163.8	3	171.5	3	171.4	3	312.5	3
222.8	4	136.6	4	166	4	174.5	4	173.5	4	314.5	4
226.9	5	142.4	5	167.8	5			175.6	5	316.6	5
228.7	6	144.4	6	170	6			177.6	6	318.8	6
		146.3	7	171.7	7			180.1	7	320.7	7
				174	8			182.1	8		
				176	9						
				187.5	10						
				189.4	11						
				191.4	12						

Table 1. Fragment size list of 57 rice cultivars analyzed with SSR markers

NO	Cultivars	CODE					
		A marker	B marker	C marker	D marker	E marker	F marker
1	광안벼	1	2	6	3	3	5
2	금안벼	2	2	9	3	5	1
3	남평벼	1	2	4	3	2	7
4	내풍벼	1	2	4	1	8	4
5	대안벼	5	4	4	3	6	2
6	대진벼	1	2	6	3	8	2
7	대평벼	2	2	11	3	6	3
8	동안벼	1	3	11	3	1	1
9	동진1호	2	2	11	3	3	3
10	동진벼	5	2	4	3	6	1
11	만금벼	5	2	4	3	2	7
12	만월벼	2	2	11	5	3	3
13	만흔벼	1	6	11	3	2	1
14	문장벼	5	6	12	2	5	1
15	삼광벼	1	2	11	3	3	3
16	삼덕벼	5	2	11	4	3	3
17	삼백벼	5	3	2	3	2	1
18	삼천벼	5	2	11	2	6	4
19	삼평벼	2	2	10	1	3	1
20	삼미벼	3	3	8	3	2	1

국내재배 57종 DB화 완료

Literature Cited

1. Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwonges J, Wu K, Sian Z, Yu Z, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksly SD 1994. Saturated molecular map of the rice genome based on an interspecific backcross population. *Genetics* **138**: 1251-1274

2. Ji HS, Koh HJ, Park SU, McCouch SR 1998. Varietal identification in japonica rice using microsatellite DNA markers *Korean J. Breed.* **30**(4):350-360.

3. Kwon SJ, Ahn SN, Hong HC, Kim YK, Hwang HG, Choi HC, Moon HP 1999. Genetic diversity of Korean japonica rice cultivars. *Korean J. Breed.* **31**(3):268-275