Cancer Chemother Pharmacol (2004) 54 (Suppl 1): S21–S24
DOI 10.1007/s00280-004-0882-2

**Naotsugu Haraguchi · Hiroshi Inoue · Koshi Mimori
Fumiaki Tanaka · Tohru Utsunomiya · Kouji Yoshikawa
Masaki Mori**

# Analysis of gastric cancer with cDNA microarray

Published online: 7 August 2004


**Abstract** Recent advances in cDNA microarray techniques have enabled us to study the expression of many genes simultaneously. As gastric cancer remains one of the most common cancers in Japan, we studied gene expression profiles in gastric cancer by cDNA microarray analysis to determine if it would be clinically useful. We demonstrated two points. First, cDNA microarray might be useful as a prognostic indicator. However, there remain several important problems to be solved and these are discussed. Second, laser microdissection plus cDNA microarray might be useful in determining the specific genes that correlate to cancer metastasis or histological subtype. We review the pros and cons for cDNA microarray analysis in the context of gastric cancer.

**Keywords** cDNA microarray · Gastric cancer · Prognosis · Metastasis · Laser microdissection

## Introduction

In this paper we discuss two topics. The first is the development of a scoring system using cDNA microarray to estimate prognosis in gastric cancer patients more comprehensively. However, this technique was found to be associated with several practical problems, such as sample preparation, which are discussed. The second topic is the use of laser microdissection and cDNA microarray analysis to identify genes related to metastasis and histological differentiation of gastric cancer.

This work was presented at the 19th Bristol-Myers Squibb Nagoya International Cancer Treatment Symposium, "State of the Arts for Digestive Organs", 14–15 November 2003, Nagoya, Japan.

N. Haraguchi · H. Inoue · K. Mimori · F. Tanaka
T. Utsunomiya · K. Yoshikawa · M. Mori (✉)
Department of Surgery and Molecular Oncology,
Medical Institute of Bioregulation, Kyushu University,
4546 Tsurumihara, 874-0838 Beppu, Japan
E-mail: mmori@beppu.kyushu-u.ac.jp
Tel.: +81-977-271645
Fax: +81-977-271651

## cDNA microarray as a prognostic indicator

We have previously studied whether cDNA microarray would be useful as a prognostic indicator [4]. Briefly, we prepared tumor/normal paired samples obtained from 43 patients with primary gastric cancer. cDNA microarray analysis was performed with commercially available DNA chips purchased from Takara Shuzo Company, Japan. The DNA chips contained 425 cancer-associated genes. The 43 patients were classified into two subgroups (aggressive or nonaggressive disease) with respect to five conventional prognostic factors: tumor size, depth of tumor invasion in the wall, histological growth pattern, lymph node metastasis, and liver metastasis.

Examination of the most differentially expressed genes in the shallow invasion vs the deep invasion tumor groups showed that matrix metalloproteinase 7, keratin 6B, thrombospondin 2, matrix metalloproteinase 10, IGF binding protein 3, and others were expressed approximately two to four times more frequently in the deep invasion group than in the shallow invasion group. The most differentially expressed genes between lymph node metastasis-negative vs lymph node metastasis-positive groups included three types of the matrix metalloproteinase family including matrix metalloproteinase 7 [3], and other genes of interest such as secreted protein acidic and rich in cystein (SPARC). SPARC is a small extra matrix-associated protein. We have previously demonstrated that production of SPARC increases during angiogenesis and enhances matrix metalloproteinase 2 expression [8]. SPARC was associated significantly with lymph node metastasis and a poorer prognosis in esophageal cancer. This was also confirmed for gastric cancer [8]. Expression of these genes was about two to three

times greater in lymph node metastasis-positive patients than in patients who were lymph node metastasis negative.

To establish a prognostic scoring system, the frequency of detection of each gene in relation to the five conventional prognostic factors was evaluated as a coefficient. If the gene was associated with all five conventional prognostic factors, its coefficient was evaluated as five. If it was associated with three conventional prognostic factors, its coefficient was three. If the gene expression was inversely correlated with the malignant potential, a minus point was given as shown by integrin beta 4. Interestingly, 347 of 425 evaluated genes were not differentially expressed between each of the two subgroups of the five conventional prognostic factors, and the coefficient was therefore zero. To calculate the prognostic score, the following formula was used: $\sum X_n Y_n$, where X represents the log value of the expression ratio of the gene and Y represents a coefficient for the gene.

Figure 1 shows the results of each patient's prognostic score, stage of disease, and outcome after operation. Of 17 patients in group A who were alive with no evidence of recurrence more than 5 years after operation, 16 scored less than 100 points. On the other hand, all 20 patients who died of recurrence within 5 years after operation scored more than 100 points. Interestingly, one patient in this group with stage I disease died of liver metastasis. The patient was classified as TNM stage I, and would have been expected to have a good prognosis, but the actual outcome was an unexpectedly early death. This suggests that the prognostic score might be better than the conventional staging system.

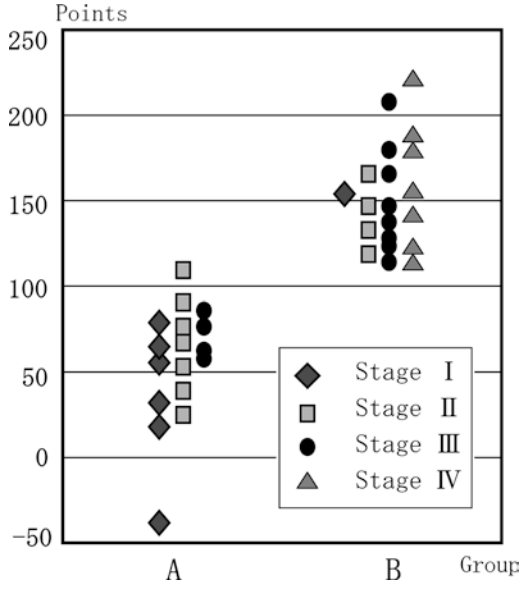


**Fig. 1** Prognostic score and prognosis in each stage of the disease. Group A included those alive and well for more than 5 years after operation. Group B included those who died of disease within 5 years of operation. All except one stage II patient in group A scored less than 100 points, while all patients in group B scored more than 100 points

## Problems of sample preparations for cDNA microarray analysis

On the basis of the above results, we tested this system in samples from eight patients from group A (good prognosis), and ten patients from group B (poor prognosis) (Fig. 2). Six of the eight group A patients scored less than 100 points, but two scored more than 100 points. On the other hand, six of the ten group B patients scored more than 100 points, but the remaining four scored less than 100 points. Thus the sensitivity of the system was 60%, and the specificity was 75%. This means that the scoring system would not be good enough to determine the prognosis of each patient.

There are considered to be two main reasons to explain the level of specificity of this prognostic scoring system. First, whether the sample is prepared from whole tumor tissue containing both tumor and stromal cells, or from tumor cells alone obtained by microdissection. The second consideration concerns which area of the tumor is obtained—the surface or advancing area. The tumor itself shows heterogeneity, so different areas may show different gene expression patterns.

We reviewed the reports of several representative studies to see which sample the authors used. Van't Veer et al. reported that a gene expression signature strongly predicted a short time to distant metastases in patients without tumor cells in the local lymph nodes at diagnosis [7]. Similarly van de Vijver et al. reported that the gene expression profile was a powerful predictor of the outcome of disease in a series of 295 patients [6]. The samples used in the two studies were bulk tissue containing both

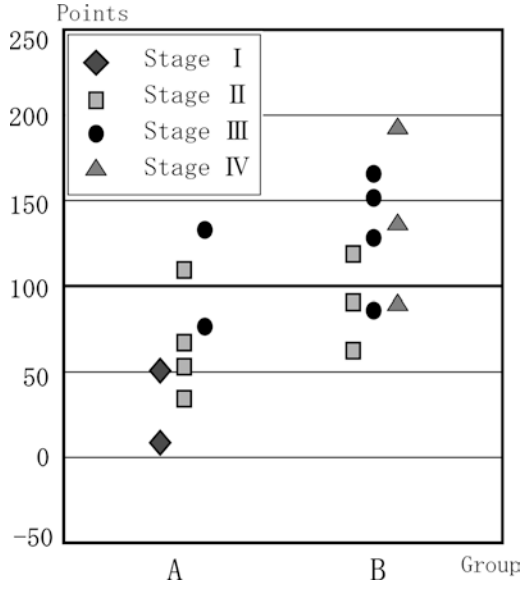


**Fig. 2** Prognostic score and prognosis at each disease stage

cancer and stromal cells. The authors confirmed that the samples contained a tumor cellularity greater than 50% by histological examination. Beer et al. demonstrated that the expression profile of 50 identified genes could predict patient survival in early-stage lung cancer [1]. The authors used bulk samples and confirmed that each sample contained a tumor cellularity greater than 70%.

On the other hand, other recent studies have indicated the importance of preparing only cancer cells by laser microdissection. Hasegawa et al. studied the prediction of lymph node metastasis in intestinal-type gastric cancer [2]. From 23,000 genes, the authors identified 12 genes that related to lymph node metastasis, and finally selected 5 genes that could determine lymph node metastasis. We must clarify which samples should be used for practical clinical analysis in the future.

The second problem concerns which area of the tumor should be used for the analysis. We compared the gene expression profiles of biopsy samples vs surface samples, and biopsy samples vs advancing area samples (data not shown). Well-differentiated cancers showed a good correlation between biopsy sample and surface sample, and between biopsy sample and advancing area sample. On the other hand, poorly differentiated cancers showed a poor correlation between biopsy samples and advancing area samples. The results indicate that well-differentiated cancers may not change their gene expression profile during tumor progression. However, poorly differentiated cancers may change their gene expression profile during tumor progression. Therefore, particularly in poorly differentiated cancers, it would be important to know from which area of the tumor the samples were extracted.

In summary, cDNA microarray analysis of clinical samples could open up a new window for cancer diagnosis; however, we must pay more attention to the practical problems such as sample preparation.

## Identification of genes related to metastasis using laser microdissection and cDNA microarray analysis

To identify metastasis-related genes, we prepared samples of primary gastric cancer and lymph node metastases. The cancer cells were laser microdissected from the primary tumor and the metastatic lymph nodes, respectively (Fig. 3) [5]. From each sample, 10 to 20 frozen sections of 8-μm thickness were prepared. As the amount of extracted RNA was small, T7 amplification was performed. Then cDNA microarray was performed and the data were compared between primary and metastatic cancer cells.

The genes overexpressed in metastatic cancer cells compared to primary cancer cells included cyclin-dependent kinase 4, fibroblast growth factor 2, epidermal growth factor, cathepsin B, and Rho-associated coiled-coil forming protein kinase (ROCK), which is associated with cell motility. Through a differential display study, we confirmed that platelet-derived endothelial cell growth factor (PDECGF) is one of the abundantly expressed genes in tumor tissue, and noted an intimate correlation between PDECGF and

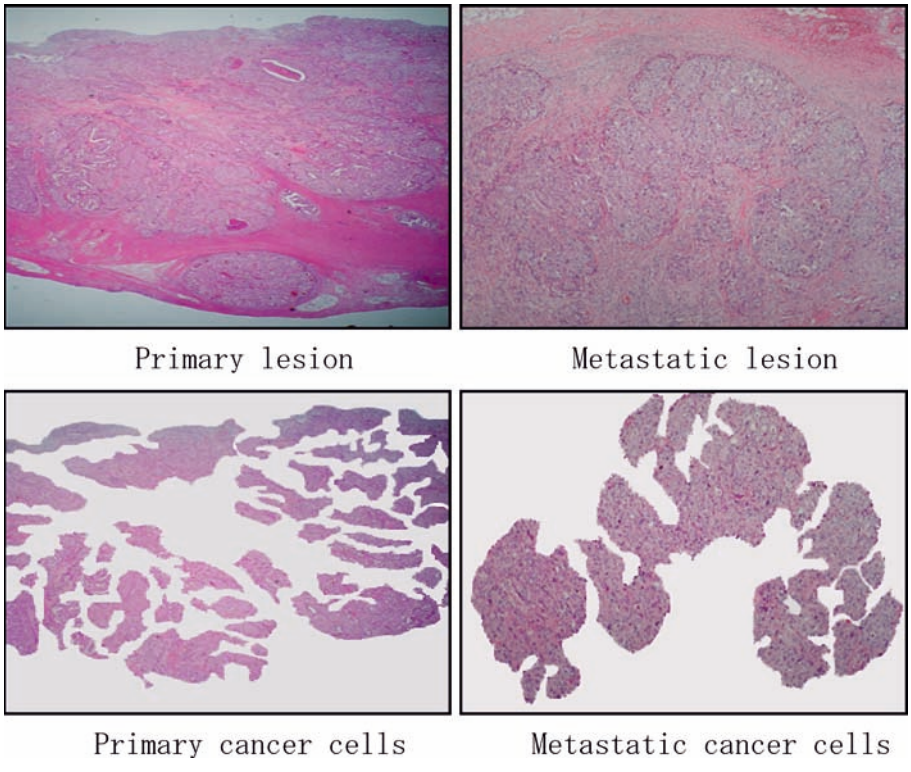


**Fig. 3** Primary cancer of the stomach and metastatic cancer in the lymph node. The lower panels show laser microdissected specimens

ROCK [9]. PDECGF is a known angiogenic factor that is identical to thymidine phosphorylase. We and other groups have demonstrated that it is overexpressed in several types of malignant tissue, showing an association with distant metastasis and prognosis.

We established three PDECGF transfectants and performed cDNA microarray studies in control cells and the three transfectants to evaluate the genes that correlate with PDECGF. ROCK was one of the genes best correlated with PDECGF. There was a statistically significant difference between the mock and transfectant with respect to the average number of migratory cells. The PDECGF transfectant with high expression of ROCK showed the highest migratory ability. The number of migratory cells counted under several conditions demonstrated that the migratory activity was increased in the presence of recombinant PDECGF, and this activity was clearly inhibited by PDECGF neutralizing antibody and ROCK inhibitor. Thus it is possible that PDECGF contributes to high-grade metastasis by mediating ROCK.

## Conclusion

The technique of cDNA microarray has brought about great advances in cancer research. However, we must be aware that there remain major problems to be solved before its practical clinical use. Analysis with laser microdissection plus cDNA microarray could be applicable to many types of studies, such as the identification of differentially expressed genes between well and poorly differentiated adenocarcinomas. This methodology could contribute to the integrated study of pathology and molecular biology.

## References


1. Beer DG, Kardia SL, Huang CC, Giordano TJ, Levin AM, Misek DE, Lin L, Chen G, Gharib TG, Thomas DG, Lizyness ML, Kuick R, Hayasaka S, Taylor JM, Iannettoni MD, Orringer MB, Hanash S (2002) Gene-expression profiles predict survival of patients with lung adenocarcinoma. Nat Med 8:816
2. Hasegawa S, Furukawa Y, Li M, Satoh S, Kato T, Watanabe T, Katagiri T, Tsunoda T, Yamaoka Y, Nakamura Y (2002) Genome-wide analysis of gene expression in intestinal-type gastric cancers using a complementary DNA microarray representing 23,040 genes. Cancer Res 62:7012
3. Honda M, Mori M, Ueo H, Sugimachi K, Akiyoshi T (1996) Matrix metalloproteinase-7 expression in gastric carcinoma. Gut 39:444
4. Inoue H, Matsuyama A, Mimori K, Ueo H, Mori M (2002) Prognostic score of gastric cancer determined by cDNA microarray. Clin Cancer Res 8:3475
5. Mori M, Mimori K, Yoshikawa Y, Shibuta K, Utsunomiya T, Sadanaga N, Tanaka F, Matsuyama A, Inoue H, Sugimachi K (2002) Analysis of the gene-expression profile regarding the progression of human gastric carcinoma. Surgery 131:S39
6. van de Vijver MJ, He YD, van 't Veer LJ, Dai H, Hart AAM, Voskuil DW, Schreiber GJ, Peterse JL, Roberts C, Marton MJ, Parrish M, Atsma D, Witteveen A, Glas A, Delahaye L, van der Velde T, Bartelink H, Rodenhuis S, Rutgers ET, Friend SH, Bernards R (2002) A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 347:1999
7. van't Veer LJ, Dai HY, van de Vijver MJ, He YDD, Hart AAM, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernards R, Friend SH (2002) Gene expression profiling predicts clinical outcome of breast cancer. Nature 415:530
8. Yamashita K, Upadhay S, Mimori K, Inoue H, Mori M (2003) Clinical significance of secreted protein acidic and rich in cystein in esophageal carcinoma and its relation to carcinoma progression. Cancer 97:2412
9. Yoshinaga K, Inoue H, Tanaka F, Mimori K, Utsunomiya T, Mori M (2003) Platelet-derived endothelial cell growth factor mediates Rho-associated coiled-coil dominant kinase messenger RNA expression and promotes cell motility. Ann Surg Oncol 10:682