



Annie Toja. *Villefranche, France, Fishing Boats*. Acrylic.

Gene expression profiling is a powerful tool that shows promise in the routine management of cancer patients.

The Potential Role of Gene Expression in the Management of Primary and Metastatic Colorectal Cancer

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Background: *Although there have been recent advances in treatment of colorectal cancer, microarray technology has the potential to improve the application of these therapies by interrogating tumor-specific molecular “fingerprints.”*

Methods: *The future applications and benefits of gene expression profiling are discussed.*

Results: *Potential uses include determining who will benefit from chemotherapy, further classifying patients into responders and nonresponders, predicting apoptotic response, developing classifiers to recognize chemosensitive tumors, identifying genes that portend a poor prognosis, revealing genes associated with metastases, predicting the outcome according to clinical stage, and avoiding surgery in patients who would not benefit from resection.*

Conclusions: *Recent research has been aimed at not only finding new molecularly targeted agents, but also identifying specific signatures to predict sensitivity and resistance to therapy. Future care may soon incorporate the data derived from a single microarray chip or similar technology that will describe a patient’s tumor, predict prognosis, and direct specific therapy.*

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Abbreviations used in this paper: mRNA = messenger RNA, cDNA = complementary DNA.

Introduction

Of the nearly 150,000 new cases of colorectal carcinoma that will be diagnosed in the United States this year, up to 50% will develop liver metastases at some point during their lifetime.¹ If left untreated, these patients have a poor median survival that ranges from 4 to 21 months and a 5-year survival that approaches zero.² Currently, the best curative therapy that can be offered is surgical resection. Unfortunately, only approximately 10% to 20% of patients with colorectal metastases are eligible for curative surgical resection. It is well

documented in the literature that the 5-year survival rate following resection for colorectal liver metastases ranges from 25% to 58%.³⁻⁶ Researchers continue to explore methods to increase survival by preventing metastases with adjuvant therapy and downstaging unresectable metastases with combination chemotherapy in order to allow surgical resection. Significant efforts are underway not only to find new agents that will effectively treat these malignancies, but also to develop strategies for predicting which patients will respond to these agents. Microarray gene-expression analysis is a promising new tool that may help to identify specific biological characteristics of how the cancer behaves, which in turn could help direct and individualize treatments by matching the right cure with the right patient at the right time.

The cell machinery transcribes messenger RNA (mRNA) from DNA when it needs to produce proteins for cellular functions. The mRNA is then translated into proteins, and the amount of RNA reflects how much protein is made. A single experiment that utilizes microarray analysis of RNA can now monitor thousands of genes at one time. The data obtained from this experiment yield a snapshot of the events taking place at the molecular level, allowing for investigations into which genes may be upregulated or downregulated. This specific "profile" can then be used to define or describe different tissues, normal or neoplastic.

Recent advances in genomic science have allowed scientists to construct gene-expression profiles using large-scale microarray analyses of different tumor types. Two common array formats are available for investigating the relative expression level of individual genes: complementary DNA (cDNA)-spotted arrays and oligonucleotide-based arrays. Oligonucleotide arrays use small DNA sequences, ranging from 20 to 70 base pairs in length, that can recognize individual genes. The second format, cDNA, utilizes longer portions of DNA, representing individual genes. Small amounts of RNA, in the range of 2 to 10 µg, are isolated from the tumor as well as the normal tissue and are then converted to the more stable cDNA (or cRNA for oligonucleotide array formats) labeled with fluorescence. The cDNA/cRNA is then hybridized to the target chip from either of the two formats. In theory, if a gene is overexpressed, more cDNA/cRNA from the tumor will then be available to hybridize the microarray chip. Fluorescence is detected from the target chip, which is translated into relative levels of gene expression.

Potential Benefits and Problems

Like computer chips, microarray chips have become denser with time. Today's microarray chips are capa-

ble of examining upwards of 50,000 different genetic elements on a single microscope slide. Originally, these high-density arrays were designed for gene discovery. Research is now directed towards using these chips to identify a unique clinical problem or to understand the complex relationships expressed between genes. However, this process should not be oversimplified. There are numerous steps during gene expression analysis that have been refined over the years as well as extensive research involved with both validation and interpretation of this technique. For example, new techniques have evolved regarding the heterogeneity of the sample under investigation. Laser capture microdissection is a method developed to target specific cells in a multi-populated tissue sample. This dissecting tool is used to capture the desired cells for analysis while leaving the remaining adjacent tissue undisturbed for future investigation.⁷ In addition to sample procurement, error may arise from the steps involved with hybridization and fluorescence detection. Normalization and quality control are necessary if data are to be reproducible and shared between different laboratories using different platforms. Gene expression profiling allows for an enormous amount of data from tens of thousands of genes. This is where the formulation of statistical algorithms has been essential for data analysis. A large amount of time and effort has gone into deriving these sophisticated analytical algorithms and developing methods for successful interpretation, bearing in mind that because a gene is upregulated does not mean that it is directly involved with the biological process in question.⁸ In addition, it must be recognized that gene expression profiling is based on measuring levels of mRNA, and these levels do not necessarily correlate with protein levels. Furthermore, posttranslational modification of these proteins may play an important role in tumorigenesis, which has led researchers to explore the field of proteomics and to design protein microarrays to reveal protein expression and activity.

Despite these potential problem areas, we envision that specific chips will be available for different tumor types and also that a single chip may possibly be used to target more than one clinical problem. One set of genes might be useful for differential diagnosis, while another set could focus on predicting prognosis. Similarly, a different set of genes altogether might be used to address sensitivity to therapy. This would be a multifunctioning chip for a specific set of clinical questions. The treatment of colorectal cancer may benefit from all of these potential roles for gene expression profiling — better classification of tumor types, response to chemotherapy (for preventing metastases and for downstaging existing metastases), and identification of patients who would benefit from further surgical treatments.

Patient Selection

Although chemotherapy for metastatic colon cancer continues to improve, the response rates remain at 34% to 50% at best.⁹ Adam et al¹⁰ reported on their experience of 701 patients with unresectable liver metastases. Following neoadjuvant chemotherapy, 95 patients (13.5 %) were deemed candidates for curative resection. This study showed that effective chemotherapy could allow a sizable number of additional patients to go on to curative resection, and molecular profiling may be a way to determine which patients will actually benefit from these chemotherapies. Currently, the pathologist uses histopathologic techniques and immunohistochemical analyses to “classify” the tumors. However, the use of specific genetic “fingerprints” could potentially allow further classification of patients into groups of responders and nonresponders, thus saving the nonresponders from the toxic side effects of chemotherapy. Arango et al¹¹ studied the expression profile of 30 different colorectal cancer cell lines. By utilizing a 9216-sequence cDNA microarray, they were able to predict the apoptotic response to a specific chemotherapeutic agent. They also utilized the expression profile of 30 different colon carcinoma cell lines to predict the response to 5-fluorouracil and camptothecin. They were able to show that 50 genes selected together were better at predicting response than four previously utilized identification markers: thymidylate synthase and thymidine phosphorylase activity, p53 status, and mismatch repair status.¹²

Apoptosis plays an important role in response to both radiation and chemotherapy. Defects in the apoptosis machinery may lead to poorer outcomes by allowing metastatic spread¹³ or resistance to therapy. Krajewska et al¹⁴ used tissue microarray to identify specific apoptotic regulators that correlated with poorer outcomes in stage II colorectal cancers. Likewise, microarray analysis has been used to identify a set of genes responsible for resistance to apoptosis following treatment with cisplatin,¹⁵ potentially identifying new genetic targets to help improve the effectiveness of chemotherapy.

At our institute, we recently initiated a phase II clinical colorectal cancer trial to specifically develop effective molecular classifiers to recognize chemosensitive tumors. Patients with colorectal metastases will initially undergo core needle biopsies for diagnosis and tissue sampling. These tissue samples will then be used to extract RNA and to identify molecular fingerprints that will be able to predict response to two specific chemotherapy regimens. More information on this clinical trial is available at the Florida Cancer Trials Web site (<http://www.floridacancertrials.com>). Previously published classifiers have evaluated a relatively small number of tumors and/or tumor types, and their accu-

racy in predicting tumor classification has not met the accuracy required for clinical application.¹⁶ Our own preliminary studies have provided data that demonstrate a family of multiclass tissue classifiers with previously unreported levels of accuracy. Utilizing a spotted cDNA microarray containing approximately 32,000 elements, in addition to data produced within the oligonucleotide platform, we were able to generate a classifier with 90% accuracy.

To better identify patients with colon cancer who are at risk for disease progression and metastasis, we and others have sought to identify genes that portend a poor prognosis. We recently utilized gene expression arrays in the investigation of metastatic potential of selected human colon cancer tumor cell lines in a nude mouse model.¹⁷ By choosing three poorly metastatic tumor cell lines and three highly metastatic tumor cell lines, we were able to identify those genes that may be linked to a tumor's metastatic potential. Approximately 100 individual genes that may predict metastatic potential were identified.

Similar experiments have also revealed potential genes associated with metastases. Li et al¹⁸ analyzed the expression profiles of 14 primary colorectal cancers with liver metastases. They compared these to 11 non-metastatic carcinomas as well as 9 adenomas of the colon. Although a number of genes were upregulated in both metastatic and nonmetastatic carcinomas, the authors identified 53 upregulated genes frequently associated with primary tumors with metastases. They also identified 375 genes that were downregulated in these same tumors. Bandres et al¹⁹ utilized cDNA microarray to detect differences in gene expression profiles among colon tumors of 20 different patients. Cluster analysis revealed two separate expression profiles that differentiated those tumors with or without lymph node involvement. Again, genes involved with apoptotic pathways were identified as potential regulators of nodal metastases.

Genes Associated With Metastasis

More recently, we have developed a molecular signature of 43 genes that has promise in predicting the outcome for clinical stages. Eschrich et al²⁰ recently demonstrated that a 43-gene signature was capable of discriminating good from poor prognosis patients within a 90% accuracy. Fig 1 shows these results with both cluster analysis and survival curves revealing the potential for predicting outcomes utilizing this classifier. The genes associated with the poor prognosis group may be linked to the process of liver metastasis.

Microarray analysis could also be used to predict which patients would actually benefit from surgical resection of liver metastases. Previously, many factors

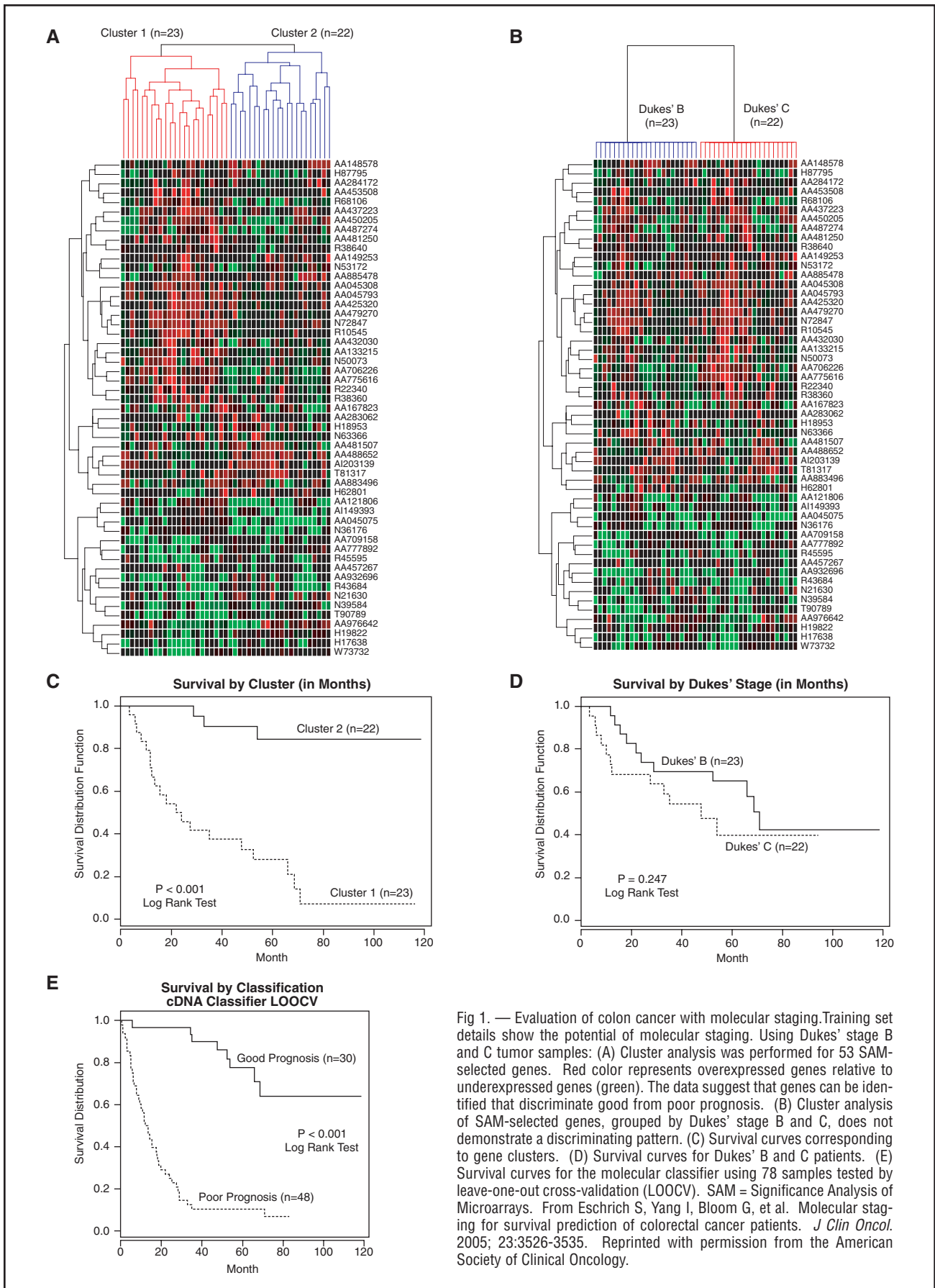


Fig 1. — Evaluation of colon cancer with molecular staging. Training set details show the potential of molecular staging. Using Dukes' stage B and C tumor samples: (A) Cluster analysis was performed for 53 SAM-selected genes. Red color represents overexpressed genes relative to underexpressed genes (green). The data suggest that genes can be identified that discriminate good from poor prognosis. (B) Cluster analysis of SAM-selected genes, grouped by Dukes' stage B and C, does not demonstrate a discriminating pattern. (C) Survival curves corresponding to gene clusters. (D) Survival curves for Dukes' B and C patients. (E) Survival curves for the molecular classifier using 78 samples tested by leave-one-out cross-validation (LOOCV). SAM = Significance Analysis of Microarrays. From Eschrich S, Yang I, Bloom G, et al. Molecular staging for survival prediction of colorectal cancer patients. *J Clin Oncol*. 2005; 23:3526-3535. Reprinted with permission from the American Society of Clinical Oncology.

were considered when determining who would have a favorable outcome following hepatic resection. A clinical risk score as described by Fong et al⁴ utilizes lymph node status from primary resection, disease-free interval between colon and liver disease, size of tumor, number of liver tumors, and carcinoembryonic antigen level to help select those who would benefit most from surgical intervention. By incorporating genetic analysis, we may be able to define these populations even further to identify a good prognosis subpopulation among metastatic patients. There may be specific genetic fingerprints that describe poor outcome and allow these patients to forgo potential complications associated with major surgery.

Conclusions

Genetic expression profiling may help direct future therapies in the treatment of hepatic metastases of primary colorectal tumors. By identifying specific genes involved with tumor progression as well as those responsible for treatment resistance, physicians will be able to focus therapies on those who will achieve the most benefit. Also, new molecular targets may be discovered. Molecular analysis will play a major role in the comprehensive treatment of cancer in the near future and will change cancer management as we know it from a defensive, overtreatment approach to a more targeted, personalized approach.

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References

1. Steele G, Jr, Ravikumar TS. Resection of hepatic metastases from colorectal cancer. Biologic perspective. *Ann Surg.* 1989;210:127-138.
2. Scheele J, Stangl R, Altendorf-Hofmann A. Hepatic metastases from colorectal carcinoma: impact of surgical resection on the natural history. *Br J Surg.* 1990;77:1241-1246.
3. Yamaguchi A, Kurosaka Y, Kanno M, et al. Analysis of hepatic recurrence of colorectal cancer after resection of hepatic metastases. *Int Surg.* 1993;78:16-19.
4. Fong Y, Fortner J, Sun RL, et al. Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases. *Ann Surg.* 1999;230:309-321.
5. Jenkins LT, Millikan KW, Bines SD, et al. Hepatic resection for metastatic colorectal cancer. *Am Surg.* 1997;63:605-610.
6. Fernandez FG, Drebin JA, Linehan DC, et al. Five-year survival after resection of hepatic metastases from colorectal cancer in patients screened by positron emission tomography with F-18 fluorodeoxyglucose (FDG-PET). *Ann Surg.* 2004;240:438-450.
7. Bonner RF, Emmert-Buck M, Cole K, et al. Laser capture microdissection: molecular analysis of tissue. *Science.* 1997;278:1481-1483.
8. King HC, Sinha AA. Gene expression profile analysis by DNA microarrays: promise and pitfalls. *JAMA.* 2001;286:2280-2288.
9. Hurwitz H. Integrating the anti-VEGF-A humanized monoclonal antibody bevacizumab with chemotherapy in advanced colorectal cancer. *Clin Colorectal Cancer.* 2004;4(suppl 2):S62-S68.
10. Adam R, Avisar E, Ariche A, et al. Five-year survival following hepatic resection after neoadjuvant therapy for nonresectable colorectal. *Ann Surg Oncol.* 2001;8:347-353.
11. Arango D, Wilson AJ, Shi Q, et al. Molecular mechanisms of action and prediction of response to oxaliplatin in colorectal cancer cells. *Br J Cancer.* 2004;91:1931-1946.
12. Mariadason JM, Arango D, Shi Q, et al. Gene expression profiling-based prediction of response of colon carcinoma cells to 5-fluorouracil and camptothecin. *Cancer Res.* 2003;63:8791-8812.
13. Compagni A, Christofori G. Recent advances in research on multi-stage tumorigenesis. *Br J Cancer.* 2000;83:1-5.
14. Krajewska M, Kim H, Kim C, et al. Analysis of apoptosis protein expression in early-stage colorectal cancer suggests opportunities for new prognostic biomarkers. *Clin Cancer Res.* 2005;11:5451-5461.
15. Huerta S, Harris DM, Jazirehi A, et al. Gene expression profile of metastatic colon cancer cells resistant to cisplatin-induced apoptosis. *Int J Oncol.* 2003;22:663-670.
16. Ramaswamy S, Tamayo P, Rifkin R, et al. Multiclass cancer diagnosis using tumor gene expression signatures. *Proc Natl Acad Sci U S A.* 2001;98:15149-15154. Epub 2001 Dec 11.
17. Hegde P, Qi R, Gaspard R, et al. Identification of tumor markers in models of human colorectal cancer using a 19,200-element complementary DNA microarray. *Cancer Res.* 2001;61:7792-7797.
18. Li M, Lin YM, Hasegawa S, et al. Genes associated with liver metastasis of colon cancer, identified by genome-wide cDNA microarray. *Int J Oncol.* 2004;24:305-312.
19. Bandres E, Catalan V, Sola I, et al. Dysregulation of apoptosis is a major mechanism in the lymph node involvement in colorectal carcinoma. *Oncol Rep.* 2004;12:287-292.
20. Eschrich S, Yang I, Bloom G, et al. Molecular staging for survival prediction of colorectal cancer patients. *J Clin Oncol.* 2005;23:3526-3535.