



Jennifer Eisenpresser. *Jade Garden*. Oil on wood panel, 16.5" × 20".

Microarray technology identifies global gene expression alterations that are involved in the development of melanoma.

The Promise of Microarray Technology in Melanoma Care

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Background: Genetic aberration is responsible for the development of neoplastic potential in a number of malignancies. These DNA alterations result in significant changes in gene expression that may now be measured and catalogued. The microarray technique screens and identifies expressed genes that may be responsible for tumorigenesis.

Methods: The authors review the application of the microarray technique in malignant melanoma.

Results: Candidate melanoma suppressor genes have been identified in melanoma cell lines using this technique. Furthermore, molecular classification using gene expression profiling may improve the accuracy of the staging system for determining prognosis.

Conclusions: The microarray technique is in its initial development for clinical application in a variety of tumor models. Melanoma is an ideal system to study the genetic changes associated with the stepwise progression of malignancy. It may be possible to efficiently screen the entire human genome to identify the particular aberrations in gene expression responsible for tumorigenesis in melanoma.

Introduction

Each human cell contains approximately 3 billion DNA base pairs that recently have been estimated to encode approximately 30,000 genes responsible for maintaining the structure and function of a cell.¹ These genes encode the RNA and proteins that produce the phenotype of the cell. The development of the neoplastic phenotype occurs as a result of an aberration or alteration in the expression of these normal genes. A variety of specific mechanisms have been implicated in the transformation process including chromosomal

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rearrangement, deletions, amplification, and mutations of genes. Elucidating the fundamental molecular mechanisms that are involved in the stepwise progression from normal tissues to malignant tumors is essential in our knowledge of cancers. This would ultimately lead to improved methods of detection, treatment, and cures for cancers.

Melanoma is ideal for the study of mechanisms involved in the stepwise progression of disease from normal pigment cells to atypical nevi to invasive primary melanoma and finally to cells with aggressive metastatic potential. Reports have shown that the most frequent genetic aberrations in malignant melanoma are rearrangements in chromosome 1, which harbors a tumor-suppressor gene. In addition, defects such as loss, duplication, or mutations of genes on chromosome 6, 7, 9, 10 and 11, 22, and Y have also been identified.² Abnormal proliferation and tumor progression of melanoma cells include defects in the genes of cell cycle regulatory proteins, DNA repair, cell cycle arrest, apoptosis, growth factors, and cytokines. The underlying hypothesis is that specific DNA alterations (eg, amplification, deletion, mutation) will result in significant RNA alterations that can be measured and catalogued.

Techniques for the analysis of gene expression include Northern blotting,³ differential display,⁴ serial analysis of gene expression (SAGE),⁵ and dot blot analysis.⁶ Each technique has its own drawbacks and limita-

tions and can be labor intensive with limitations of the number of samples that can be studied in one experiment. Recently discovered DNA microarray technology has begun to revolutionize the technology in gene expression analysis in a variety of human diseases⁷ and particularly in human cancers (Fig 1).⁸⁻¹⁰ Gene expression analyses will allow the identification of numerous representative novel genes that may be involved in the tumorigenesis for functional studies. With this technology, virtually thousands of genes may be examined with one experiment.

Microarray Technique

The microarray techniques, first described in 1994 by Drmanac et al,^{11,12} use the method of hybridization of large-scale cDNA with a “mass” probe to identify the expression of individual genes. This was fueled in part by the Human Genome Project and was begun in an attempt to catalog, map, and sequence all genes and define the expression in individual cells. In the authors’ initial report, microarrays containing up to 31,000 cDNA clones were PCR-amplified and spotted on nylon filters using an automated system. This technique was later improved whereby the DNA microarrays were spotted on a single microscopic glass slide⁷ and differential expression was analyzed from two different tissue samples by two-colored fluorescent labeling. A newer method by Affymetrix GeneChip

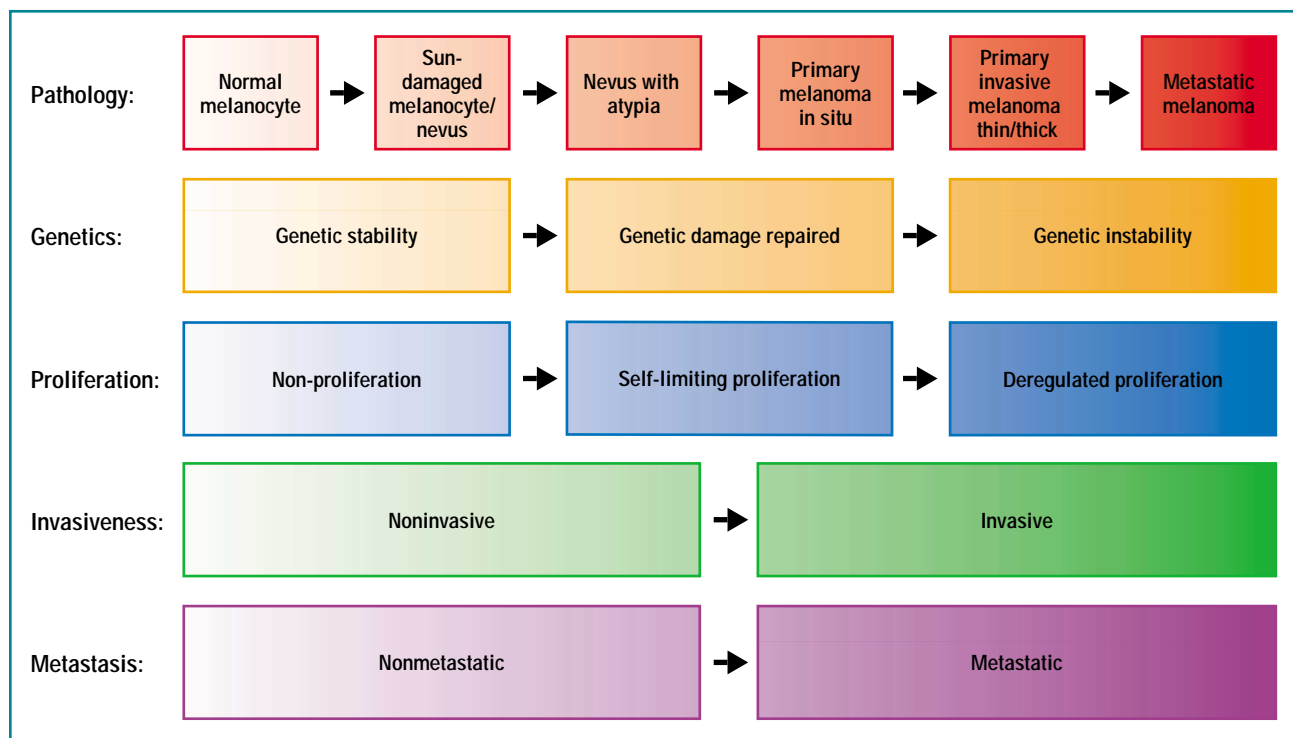


Fig 1. — Biologic alterations occurring in progression to malignant melanoma. From *Cancer: Principles & Practice of Oncology*. 5th ed. DeVita VT, Hellman S, Rosenberg SA, eds. Philadelphia, Pa: Lippincott-Raven; 1997:1937. Chapter 41. Reprinted with permission.

(Affymetrix, Inc, Santa Clara, Calif) utilizes silicon chips where more than 400,000 oligonucleotides can be synthesized on a single 1.6-cm² microscopic glass slide.

The technique is based on hybridization of mRNA or a more stable copy (cDNA) with target sequences (cDNA or oligonucleotides) that correspond to a specific gene. Each sample mRNA is labeled with a fluorescent oligonucleotide, and reverse transcription targets cDNA since cDNA is more stable. The labeled samples are then hybridized to an array of target sequences (Fig 2). Fluorescence intensities are detected and measured by a laser confocal scanning microscope. The image is analyzed using software to scan the probes. The target is identified based on the fluorescent intensity.

The major advantage of DNA microarray is the screening of large numbers of genes with greater sensitivity using a smaller amount of sample. Currently, up to 15,000 cDNA probes can be placed on a small microscope glass slide with the future goal to screen the entire human genome (approximately 30,000 expressed genes) in one experiment. A number of expressed sequence tags (ESTs) are redundant in the human genome, and efforts are ongoing to group those genes into specific categories.

In addition to mass screening of genes and the identification of novel genes of interest, the microarray technique may be valuable in studying the interactions between genes and pathways of cell signaling. It is a powerful tool in studying the effects of changes made in the cellular signaling pathways and identifying the changes that occur in the function of other genes downstream to various genetic alterations.

Microarrays in Melanoma

It is well known that genetic alterations are associated with the development of malignant melanoma.¹ The molecular and biologic mechanisms of how a normal melanocyte of adult skin transforms into a malignant melanoma cell, however, remains unclear. The molecular and biologic events in the development of melanoma serve as an excellent model in which to study the progression of cancer. With a series of mutational events, normal melanocytes are transformed into dysplastic cells that then progress to invasive melanoma cells capable of metastatic spread throughout the body. The fact that the majority of benign nevi and even dysplastic atypical nevi do not progress to malignant melanoma cells suggests that the process of tumor progression may be inefficient. We presume that multiple genetic alterations that have not yet been fully elucidated lead to complex patterns of gene

expression resulting in the transformation and progression of disease. With current advances in DNA microarray technology, regulation of gene expression in the sequence of steps that take place from normal melanocytic cell to malignant melanoma can now be studied more rapidly with greater accuracy. In the near future, a significant amount of information will become available to the clinician and the basic scientist that will allow us to investigate the process of cell development and function.¹³

High-density microarrays of DNA have been used to search for differences in gene expression associated with tumor suppression in human melanoma cell lines.¹⁴ More than 1,100 gene elements were robotically printed onto a standard glass microscope slide. Of these, several candidate genes for tumorigenic features of melanoma cell lines were identified, including mRNA, which encodes for proteins such as TRP-1, melanoma antigen gp75, monocyte chemotactic protein 1, and melanoma differentiation antigen WAF1 (p21).¹⁵⁻¹⁷ The technique was highly accurate when compared with the gene expression analysis by Northern blotting. Additional candidate melanoma suppressor genes have been identified in human melanoma cell lines by investigating the overall gene expression in

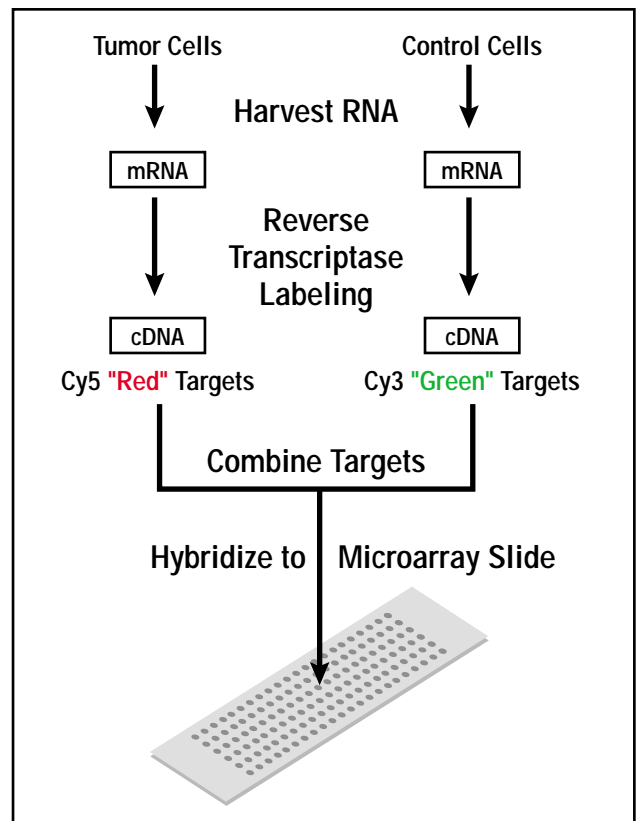


Fig 2. — The principle of gene expression analysis by DNA microarray technology. From Khan J, Saal LH, Bittner ML, et al. Expression profiling in cancer using cDNA microarrays. *Electrophoresis*. 1999;20:223-229. Reprinted with permission.

successive genetic alterations that lead to different cellular phenotypes¹⁸ using the technique of DNA microarray. Of the 3,317 genes common to three parental melanoma cell lines that were initially examined by microarray, it was possible to identify these 12 candidate genes that displayed a higher level of expression in the tumorigenic, anchorage-independent growth melanoma cell line.

A more recent study with microarray gene chip analyses compared the gene expression from melanoma cell lines derived from patients with highly aggressive and poorly aggressive uveal melanoma.¹⁹ Significant increases in gene expression of laminin-5 γ 2, matrix metalloproteinases (MMP-1, -2, -9), and MMP-14 were found in aggressive vs poorly aggressive melanoma cell lines. The study found that increases in the expression of these genes were required for "vascular mimicry" in aggressive tumor cells, a process by which tumor cells in three-dimensional matrices mimic embryonic vasculogenesis by forming an extracellular, matrix-rich, patterned tubular network. Additional studies have identified other promising genes that may be responsible for vasculogenic mimicry such as epithelial cell kinases²⁰ and VE-cadherin.²¹ Therefore, microarray gene analyses can be utilized to identify key genes that may be involved in the biology of aggressive tumors. The identification of such molecular markers may have implications for the development of novel therapies directed at genes and proteins to alter tumor progression.

Despite classification using extensive sets of histologic and immunohistochemical markers for primary melanoma, no specific independent criteria exist for predicting the prognosis in these patients. In fact, the most accurate predictor of tumor behavior relates to the thickness and ulceration of the primary tumor. Molecular classification of melanoma using gene expression profiling has been suggested as a means to improve on identifying subsets of patients with melanoma who will do well and those who have metastatic disease and are at risk for progression of their disease.²² Our analysis of gene expression profiles in 38 samples of melanoma showed clustering of 19 melanoma cell lines that could represent a new subtype of melanoma patients with a worse prognosis that had not previously been reported. The clustering of these 19 samples was also used to test for phenotypic associations with variables such as Breslow's thickness, Clark's level, patient age and sex, and site, and the genetic finding did not correlate with any of the known prognostic features for primary melanoma. The patient population in this study overall had advanced-stage melanoma with uniformly poor prognosis and therefore, no correlation could be made with clinical outcome.

More than 1 million EST sequences are currently available to an investigator in various databases representing up to 30,000 human genes. Microarray technology will likely allow a full complement of human genes to be screened for differential expression in one experiment. However, to date, one limitation of microarray technology is that the human cDNA microarray sets described are composed of a subset of the genes in the human genome.¹ It is not yet possible, even with the entire human genome sequenced, to identify the complete set of all expressed genes. To improve on this, Loftus et al²³ demonstrated a method of selection of tissue-specific EST array sets to complement more general chips that are available. A neural crest expressed gene library was used to identify differential expression of genes in melanoma cell lines compared with nonneural crest kidney epithelial cell line. These focused gene chips may permit investigators to more rapidly address mechanistic questions.

Conclusions

A complex set of genetic alterations occurs within a cell in order to permit neoplastic transformation. Melanoma is an ideal tumor model to study the molecular mechanisms of tumor progression. DNA microarray technology is a powerful tool uniquely selected for this purpose that can enable us to elucidate the exact mechanisms and defects in genetic aberrations. This technology will generate a large body of data that will require a certain structured format to permit application of this vast amount of information by investigators. Several efforts are underway toward a single site for the storage of the information as it becomes available.²⁴ With the human genome now completely sequenced, it will soon be possible to screen the entire set of human genes in a single experiment using DNA microarray techniques. These technologic advances will allow the identification of global gene expression alterations that are involved in the development of malignant melanoma. These lines of investigation hold promise in improving screening methods to identify those individuals at increased risk of developing melanoma and in developing of treatments using "gene-directed" therapy.

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