肿瘤分子学分析

Molecular Analysis of Cancer

Jacqueline Boultwood
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Preface

Over the past 20 years, technological advances in molecular biology have proven invaluable to the understanding of the pathogenesis of human cancer. The application of molecular technology to the study of cancer has not only led to advances in tumor diagnosis, but has also provided markers for the assessment of prognosis and disease progression. The aim of *Molecular Analysis of Cancer* is to provide a comprehensive collection of the most up-to-date techniques for the detection of molecular changes in human cancer. Leading researchers in the field have contributed chapters detailing practical procedures for a wide range of state-of-the-art techniques.

Molecular Analysis of Cancer includes chapters describing techniques for the identification of chromosomal abnormalities and comprising: fluorescent *in situ* hybridization (FISH), spectral karyotyping (SKY), comparative genomic hybridization (CGH), and microsatellite analysis. FISH has a prominent role in the molecular analysis of cancer and can be used for the detection of numerical and structural chromosomal abnormalities. The recently described SKY, in which all human metaphase chromosomes are visualized in specific colors, allows for the definition of all chromosomal rearrangements and marker chromosomes in a tumor cell. Protocols for the detection of chromosomal rearrangements by PCR and RT-PCR are described, as well as the technique of DNA fingerprinting, a powerful tool for studying somatic genetic alterations in tumorigenesis. A number of approaches to identify mutations are detailed, and include SSCP, DGGE, the nonisotopic RNase cleavage assay, the protein truncation assay, and DNA sequencing. A change in DNA methylation status is commonly observed in cancer, and specific methodology for methylation analysis is also provided by this volume.

The analysis of gene expression represents a key area of research in the study of human cancer and a number of chapters in *Molecular Analysis of Cancer* address this subject. Global RNA expression analysis using microarray technology allows the identification of genes that are differentially expressed in tumor versus normal tissues. This is a powerful approach for identifying genes that are central to disease development or progression and can also identify new prognostic markers.

A reduction in telomere length, together with expression of the telomere maintenance enzyme, telomerase, has been described in a wide range of human cancers. To complete the volume, we include chapters describing the measurement of telomere length and telomerase levels, an area of extensive study in the field of cancer research.

We wish to thank the authors of the various chapters of *Molecular Analysis of Cancer* for their excellent contributions. Clearly, they share our hope that this volume will assist other researchers in the analysis and detection of genetic abnormalities occurring in human malignancy, and lead to a better understanding of the molecular pathogenesis of cancer.

Jackie Boultwood
Carrie Fidler

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Contents

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Molecular Analysis of Cancer

An Overview

Ken Mills

1. Introduction

Cancer is a complex disease occurring as a result of a progressive accumulation of genetic aberrations and epigenetic changes that enable escape from normal cellular and environmental controls *(1)*. Neoplastic cells may have numerous acquired genetic abnormalities including aneuploidy, chromosomal rearrangements, amplifications, deletions, gene rearrangements, and loss-of-function or gain-of-function mutations. Recent studies have also highlighted the importance of epigenetic alterations of certain genes that result in the inactivation of their functions in some human cancers. These aberrations lead to the abnormal behavior common to all neoplastic cells: dysregulated growth, lack of contact inhibition, genomic instability, and propensity for metastasis.

The genes affected by mutations in cancer may be divided into two main classes: genes that have gain-of-function (activating) mutations, which are known as oncogenes; and genes for which both alleles have loss-of-function (inactivating) mutations, which are known as tumor suppressor genes. Close to 100 genes have been shown to play a role in the development or progression of human cancers, some of which have been implicated in a broad spectrum of malignancies, whereas others are unique to a specific type. Cancers can arise via the aberration of different combinations of genes, which in turn may be mutated, overexpressed, or deleted. The order in which these events occur has also proved to be important. For example, in breast cancer it has been proposed that at least 10 distinct gene alterations may be involved in disease initiation and progression *(2)*. The study of colon cancer has shown that carcinogenesis

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is a multistage process involving the activation of cellular oncogenes, the deletion of multiple chromosomal regions, and the loss of function of tumor suppressor genes *(3)*.

Technologic advances in molecular biology over the past 20–25 yr have led to a dramatic increase in the identification of the molecular processes involved in tumorigenesis. Over this period, the molecular basis of cancer no longer holds the mystery that it once did *(1)*. It is, however, also clear that the knowledge that has been accumulated is insufficient to claim a total understanding of the mechanism of cancer development. This volume has brought together a number of relevant techniques by which genetic abnormalities occurring in cancer can be detected and analyzed. This, in turn, will give rise to other avenues of study, such as: how mutations affect function, how these genes are regulated, and how they interact with each other.

The mutational analysis of oncogenes and tumor suppressor genes can provide evidence for a specific association between these genes and tumor type. These genes can be altered during carcinogenesis by different mechanisms such as point mutations, chromosomal translocations, gene amplification, or deletion. Furthermore, these genes may be analyzed at different levels—DNA, RNA, or expressed proteins.

2. DNA Analysis

Mutational analysis can be performed using a variety of techniques, and the majority of these are highlighted in this volume. The amplification of specific regions of DNA or RNA (Chapters 5–8) by the polymerase chain reaction (PCR) has opened endless possibilities that can be used for the rapid and efficient detection of alterations, even single nucleotide changes. These PCR-based techniques rely on changes in electrophoretic mobility induced by altered single-stranded secondary structure (single-strand conformation polymorphism) (Chapter 9), by altered dissociation rates of the DNA fragments (denaturing gradient gel electrophoresis) (Chapter 10), or by RNase cleavage assays (Chapter 11). PCR can also be used for the rapid and quantitative detection of chromosomal rearrangements, such as commonly observed in leukemia (Chapter 7). PCR is designed to specifically amplify genomic fragments that are not normally contiguous and are, therefore, unique to that type of gene rearrangement. Converting the RNA to DNA with reverse transcriptase (RT) prior to the PCR stage is usually required for this assay. However, in some cases, genomic DNA can be used for the direct amplification of translocation break points (Chapter 7). A variation on the PCR theme involves the use of DNA fingerprints to detect genetic rearrangements in cancer (Chapter 8). The primers are often arbitrary or repeat (e.g., ALU) sequences, which will give, after electrophoresis, a DNA fingerprint that can be used for the detection of genetic abnormalities. Microsatellite repeats occur throughout the

genome and can be used as markers for genetic alterations, usually for the loss of heterozygosity, which will indicate that a deletion has occurred that overlaps that specific marker (Chapter 5). For specific genes involved in certain cancers, the mutational analysis can be carried out using a protein truncation assay (Chapter 12). This assay involves the identification of abnormal polypeptides synthesized in vitro from RT-PCR products, and the truncating mutations are usually confirmed by sequence analysis.

3. RNA Expression Analysis

DNA microarray technology, which makes use of high-density two-dimensional oligonucleotide probe arrays containing hundreds or thousands of oligonucleotide probes, represents a powerful new DNA sequence analysis tool to test for a variety of genetic mutations (Chapters 15 and 16). Hybridization to cDNA microarrays allows the simultaneous parallel expression analysis of thousands of genes. High-throughput gene expression profiling increasingly is becoming a valuable method for identifying genes differentially expressed in tumor vs normal tissues. Gene expression microarrays hold great promise for studies of human tumorigenesis, and the large gene expression data sets produced have the potential to provide novel insights into fundamental cancer biology at the molecular level (Chapter 16). Indeed, cDNA microarray technology has already begun to aid in the elucidation of the genetic events underlying the initiation and progression of some human cancers. Differentially expressed genes can also be detected by other techniques such as differential display (Chapter 14), which involves a random primed RT-PCR display or fingerprint of subsets of expressed RNA, or subtractive hybridization, which involves the enrichment of genes preferentially expressed in one tissue compared with a second.

4. Chromosomal Analysis

Fluorescence *in situ* hybridization (FISH) is one of the techniques with an expanding role in the molecular analysis of cancer (Chapter 2). It can be used for the simple detection of numerical and structural chromosomal abnormalities that may occur in cancer cells and is particularly useful as a tool for the diagnosis of nonrandom translocations in leukemia and numerous other cancers. To date, most FISH studies have involved the use of single whole-chromosome or gene probes. This has been taken to new levels by the development of spectral karyotyping, which involves the hybridization of 24 fluorescently labeled chromosome painting probes to metaphase spreads in such a manner that simultaneous visualization of each of the chromosomes in a different color is accomplished (Chapter 3). Using this method, it is possible to define all chromosomal rearrangements and identify all of the marker chromosomes in

tumor cells. Comparative genomic hybridization (CGH) is a FISH-based technique that can detect gains and losses of whole chromosomes and subchromosomal regions (Chapter 4). CGH is based on a two-color, competitive FISH of differentially labeled tumor and reference DNA to normal metaphase chromosomes and can scan the whole genome without prior knowledge of specific chromosomal abnormalities.

5. Analysis of Methylation Status

Some molecular methods will analyze specific changes to the DNA structure or genomic modifications. Changes in the DNA methylation status are one of the most common detectable abnormalities in human cancer. Hypermethylation within the promoters of selected genes is especially common and is usually associated with inactivation of the involved gene or genes and may be an early event in the pathogenesis of some cancers, whereas other genes become methylated during disease progression (Chapter 18).

6. Telomere and Telomerase Activity

Telomeres are repetitive DNA sequences at chromosome ends, which are necessary for maintaining chromosomal integrity. A reduction in telomere length has been described in a wide range of human cancers, including both solid tumors and leukemias. The enzyme telomerase synthesizes *de novo* telomeric repeats and incorporates them onto the DNA 3' ends of chromosomes. Telomere shortening in normal cells is a result of DNA replication events, and reduction beyond a critical length is a signal for cellular senescence. However, the maintenance of telomere length, by the activation of the enzyme telomerase, is thought to be essential for immortalization of human cancer cells to compensate for the loss of DNA from the ends of chromosomes. Therefore, the measurement of telomere length (Chapter 20) and telomerase enzyme activity levels (Chapter 21) are important in monitoring disease progression or response to therapy. Recently, the possible manipulation of telomerase has generated some excitement as an anticancer strategy.

7. Clonal Origin of Cancer

The methods I have described allow the investigator to study the myriad of genetic alterations that can occur during the initiation, development, and progression of cancer. However, it is also possible to provide insight into the transition from somatic cell mutation to neoplasia. The clonal origin of cells can be assessed in patients with X chromosome-linked polymorphisms, taking advantage of the random inactivation of the X chromosome (Chapter 19). The inactivation is related to the differentially methylated patterns on the active and inactive X chromosomes.

8. Summary

Human cancers are generally characterized by acquisition of a series of somatic mutations. Molecular techniques, such as those described in this volume, have been used to identify a plethora of chromosomal translocations and mutations associated with carcinogenesis. The analysis and comparison of the array of genetic changes occurring in malignancy will enable a move toward a better understanding of cancer development. This will eventually lead to the development of improved therapies tailored to take into account the cytogenetic and molecular characteristics of specific human cancers.

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