The Basics of Genomic Medicine and Cancer Genomes

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In this chapter, I will explain methods of detecting genomic abnormalities in cancer based on a structural analysis of chromosomes and chromosomal abnormalities found in specific types of cancer.

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To start off, exactly what are chromosomes? A chromosome is a complex consisting of DNA located in the cell nucleus and proteins (primarily histones). A chromosome condenses DNA to 1/10,000 of its original length. There are 2 types of cell division, somatic mitosis and meiosis. Chromosomes are condensed in the metaphase of mitosis and have a morphology like that shown in the figure. During the metaphase, chromosomes align along the equatorial plane of a cell nucleus. If differential staining is performed at this point, then numerous bands are evident. Thus, chromosomes in the metaphase of mitosis are typically used to observe chromosomal morphology. Chromosomes can be stained using differential staining such as Giemsa staining to distinguish up to 2,000 patterns of bands, fluorescence in situ hybridization (FISH) to detect a hybridization probe (DNA) as a fluorescent signal, a combination of chromosome painting and multicolor fluorescence, spectral karyotyping (SKY) that stains 24 chromosomes with different colors, and array CGH that identifies a small abnormality in the copy number of several kb using a DNA chip with oligo DNA probes. Array CGH can detect amplification or deletion of various cancer-related genes at one go, and it has a high diagnostic capacity. Based on an analysis using such methods, chromosomal morphology can be categorized, in order of size, into gametes, autosomal chromosomes, sex chromosomes, short arms, long arms, telomeres, centromeres, and heterochromatin. The chromosomal makeup of an individual or cells is expressed as the karyotype.

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What I want to show you here is chronic myeloid leukemia painted using SKY. As I will explain later, the Philadelphia chromosome – a reciprocal translocation of chromosome 9 and chromosome 22 — is a chromosomal abnormality noted in over 95% of chronic myeloid leukemias. SKY is a useful method of detecting such a translocation.

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The entire human genome is around 3,000 Mb in size. Differential staining normally distinguishes around 400 bands and can distinguish up to 2,000 bands. Differential staining has a resolution of 5-

10 Mb. FISH and array CGH have a resolution of around 100 kb. Of course, these methods are far inferior to next-generation sequencing, which covers the whole genome at a resolution of singe bases. Chromosomal analysis is a method of testing that is extremely effective at detecting a disease-specific translocation such as that found in a hematopoietic malignancy or osteocartilaginous tumor, a chromosome deletion or duplication at the arm level, or aneuploidy. Compared to next-generation sequencing, chromosomal analysis is relatively simple and easy, so it can be used to detect and follow-up on residual lesions after chemotherapy. Thus, chromosomal analysis remains an essential test for the diagnosis and treatment of cancer.

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Next, I will explain about various abnormalities in chromosomes. Aneuploidy is an abnormality associated with an increase or decrease in 1-2 chromosomes instead of the normal number of 46. A well-known example is trisomy 21 noted in Down's syndrome. The condition is caused by nondisjunction during cell division. In polyploidy, somatic cells are 3n or 4n instead of 2n. Most triploidy occurs when 2 sperm fertilize 1 egg. These abnormalities occur in the germline, and most are structural chromosomal abnormalities found in an individual as a whole. In contrast, temporary chromosomal abnormalities are temporarily noted in only cancer cells or some cells as a result of irradiation, viral infection, or exposure to a chemical substance. A break occurs in a chromosome, and an abnormality occurs when a fragment reattaches. Reciprocal translocation occurs when 2 or more chromosomes break and fragments are switched and then reattached to the other chromosome. An insertion is why a fragment is attached to another chromosome. A deletion is when a fragment is lost. An inversion is when a fragment is rotated 180 degrees and reattached. A ring chromosome is where the distant ends of arms beyond the cleavage site are lost and the remaining short arm and long arm are attached to one another. An isochromosome is where one arm is a mirror image of the other. Although it is not shown in the figure, a duplication is where part of a chromosome is "duplicated" lengthwise as a result of the unequal crossover of homologous chromosomes.

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Shown here are typical temporary chromosomal abnormalities that are noted in tumors. A reciprocal translocation of chromosome 9 and chromosome 22 causes fusion of the BCR gene of chromosome 22 and the ABL gene of chromosome 9. This produces a fusion protein, tyrosine kinase, that is constantly activated, and it is the direct cause of Philadelphia chromosome-positive chronic myeloid leukemia. The drug imatinib has been developed to inhibit tyrosine kinase activity, and it is therapeutically effective. A reciprocal translocation of chromosome 8 and chromosome 14 places an enhancer of the immunoglobulin heavy chain region of chromosome 14 upstream of MYC, which is an oncogene on chromosome 8. This causes overexpression of the MYC protein, resulting in

Burkitt's B cell lymphoma.

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Various reciprocal translocations that produce oncogenic fusion proteins, as exemplified by BCR-ABL, are known to be mainly associated with hematopoietic malignancies.

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Oncogene overexpression due to enhancer translocation, as exemplified by overexpression of MYC in Burkitt's B cell lymphoma, is primarily noted in hematopoietic malignancies, as would be expected. However, such oncogenic chromosomal abnormalities are seldom found in solid epithelial tumors.

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That said, Dr. Mano, now at the National Cancer Center of Japan, and his colleagues introduced a cDNA library from lung cancer into NIH-3T3 cells and they used classic methods to search for transformable genes. Dr. Mano and his colleagues identified a new type of EML4-ALK gene. This was the first oncogenic chromosomal abnormality found in lung cancer.

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An inversion occurs in chromosome 2, fusing the EML4 gene with the ALK gene to produce an EML4-ALK fusion protein. As a result, ALK tyrosine kinase activity is constantly elevated. This chromosomal abnormality accounts for around 6% of non-small cell lung cancers, and it is effectively treated with an inhibitor of ALK tyrosine kinase. Chromosomal abnormalities were thought to be largely absent in cancers other than hematopoietic malignancies and sarcomas, but the discovery of that fusion protein showed that chromosomal abnormalities cause epithelial carcinoma.

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Following the EML4-ALK fusion gene, fusion genes such as LRIG3-ROS1 and KIF5A-RET have been identified in adenocarcinoma of the lung. KIF5A-RET accounts for around 2% of adenocarcinomas of the lung. Hopes are high that it can serve as a new therapeutic molecular target. And now this chapter has concluded.