CH2.

Slide 1

To provide genomic medicine for cancer, tumor tissue must first be biopsied and cancer cells must be collected. There are times when cells cannot be collected from tumor tissue, as I will explain in the latter half of this lecture. Recently, a method of directly collecting DNA released by tumor cells or cancer cells into the blood has been developed. This is known as liquid biopsy. Once cancer cells are collected, DNA can be extracted and a genetic analysis can be performed with a next-generation sequencer to determine whether a genetic mutation is present. Let us now look at driver mutations, which are genetic mutations associated with the growth of cancer. As an example, if gene A is present, then cancer may respond to treatment A. If gene B is present, it may respond to treatment B. If gene C is present, it may respond to treatment C. Treatment targeting genes A to C is genomic medicine for cancer. The target genes are biomarkers of treatment. Treatment can be chosen by looking for those genes with a companion diagnostic reagent or via genetic panel testing.

Slide 2

I will start by talking about driver mutations.

Slide 3

Before that, I will briefly explain cell proliferation and signalling.

Cell proliferation (cell division) occurs as a result of an extracellular growth factor (a ligand) binding to a receptor, stimulating signalling. That signal travels from a receptor on the cell membrane through the cytoplasmic matrix to the nucleus. Genes that facilitate proliferation are known as oncogenes, and genes that inhibit proliferation are known as tumor supressor genes. As shown in the slide, a growth factor binds to a receptor and signalling occurs. This causes apoptosis or it causes cancer to grow, e.g. angiogenesis, proliferation, differentiation, and metastasis. A mutation in an oncogene or tumor supressor gene results in the synthesis of an abnormal protein associated with cell proliferation or dysfunction in a protein, causing “oncogenesis.”

Slide 4

I will now explain ligands and growth factors.

A ligand is a molecule that binds to a large molecule, like a receptor, and that is responsible for transmitting information from cells to cells. It is also referred to as a first messenger. In simple terms, a ligand is a “key” that conveys information and a receptor is the “keyhole” receiving that information.

Typical ligands are as shown in the slide. As I explained earlier, EGF is a ligand that is crucial to the growth of cancer.

Slide 5

I will now explain receptors .

A receptor is the general term for proteins located on the surface of the cell membrane, in the cytoplasm, and in the nucleus. These proteins specifically recognize and bind to various physiologically active substances from outside cells and they convey information about physiologically active substances to cells and DNA. Receptor tyrosine kinases play a central role in cellular differentiation and proliferation signaling. Cell surface receptors include ion channel receptors, tyrosine kinase receptors, and G protein-coupled receptors. I will now explain tyrosine kinase receptors.

Adenosine triphosphate (ATP) is a phosphate compound that releases energy in cells for metabolic reactions such as protein synthesis.

Slide 6

I will now explain tyrosine kinases (TKs). There are around 90 TKs in humans (of those, 58 are receptor tyrosine kinases, 32 are non-receptor tyrosine kinases, and the rest are neither receptor tyrosine kinases nor non-receptor tyrosine kinases). TKs are all related to cell proliferation. TKs that are often mutated in cancer cells are shown in the slide. These TKs are targets of tyrosine kinase inhibitors. EGFR is a TK. A mutation in that TK is noted in around 20-30% of all Japanese with non-small-cell lung cancer. In contrast, gefitinib (Iressa) and erlotinib (Tarceva) are highly efficacious EGFR tyrosine kinase inhibitors (EGFR-TKIs) to treat those mutations.

VEGFR is abundantly located on the cell membrane of vascular endothelium, and it is a major pathway for growth signalling. VEGFR does not actually undergo mutation. Rather, vast amounts of VEGF (a growth factor) are secreted by cancer cells. VEGF binds to the VEGFR of vascular endothelial cells and it promotes cell proliferation, i.e. angiogenesis. Thus, a TK inhibitor is used to suppress it. Drugs targeting VEGFR and PDGFR are known as “angiogenesis inhibitors” and are currently used in routine clinical practice.

Slide 7

Shown here is a schematic diagram of phosphorylation of a typical tyrosine kinase.

As shown at the bottom of the slide, a ligand binds to a tyrosine kinase receptor to form a dimer. This causes structural changes in the tyrosine kinase receptor. The ATP-binding pocket of the intracellular kinase domain opens and ATP binds to it, causing phosphorylation. The signal from the ligand is ultimately transmitted to the nucleus.

Slide 8

I will now explain driver mutations. There are 2 types of genetic mutations in cancer. A driver mutation is a mutation in a gene that directly contributes to the development of cancer and a passenger mutation is a mutation caused by instability in a gene and is unrelated to its expression. Oncogenes and tumor supressor genes are driver mutations. The EGFR and ALK genes that I will explain later

are oncogenes that are essential to oncogenesis. In contrast, the PIK3CA gene is not essential to oncogenesis.

Slide 9

I will now explain driver mutations and various types of cancer.

EGFR, ALK, ROS1, and RET are well-known genes associated with lung cancer, and adenocarcinoma of the lung in particular. Treatment of mutations in genes other than RET is covered by National Health Insurance and is provided as part of routine clinical practice.

Slide 10

I will now explain the role of the EGFR, ALK, ROS1, and RET genes in adenocarcinoma of the lung.

Slide 11

A mutation in the EGFR gene is noted in about 50% of adenocarcinomas of the lung. An ALK fusion gene is noted in 3.8%, and a ROS1 fusion gene is noted in 0.9%. Molecularly targeted inhibitors of these 3 mutations can be used in routine clinical practice and they are highly therapeutically effective. Treatments for the BRAF and RET fusion genes are not available as part of routine clinical practice, but molecularly targeted drugs have been developed and may be used in the future.

Slide 12

There are racial differences in the incidence of driver mutations. Data for Japanese are on the left and data for Westerners are on the right. A mutation in the EGFR gene is noted in 53% of Japanese versus 11.3% of Westerners, so its incidence clearly differs.

Slide 13

Differences in the expression of driver mutations are also noted in terms of smoking history. An EGFR mutation has been detected in 58.6% of non-smokers and in 47.5% of smokers.

Slide 14

There are also sex differences in the expression of driver mutations. An EGFR mutation has been detected in 62.7% of women and in 43.0% of men.

Slide 15

The frequency of a mutation in the EGFR gene differs depending on the type of cancer. As was indicated a moment ago, a mutation has been noted at a frequency of about 50% in adenocarcinomas of the lung. As shown in the slide, a mutation has been noted in 4% of pancreatic cancers, 14% of

cholangiocarcinomas, 12% of esophageal cancers, and 1-16% of head and neck cancers, but it has not been noted in hepatocellular carcinoma, colon cancer, breast cancer, gastric cancer, or leukemia.

Slide 16

I will now explain about the EGFR gene. The EGFR gene is located on the short arm of chromosome 7 (7p12) and it consists of 28 exons and 27 introns. Exons 1-16 code for extracellular domains, exon 17 codes for a transmembrane domain, and exons 18-28 code for intracellular domains.

Slide 17

Exons 18, 19, 20, and 21 are located in the intracellular domain. Deletion of 15 bases in exon 19 results in the deletion of 5 amino acids. L858R is a point mutation where arginine is substituted for leucine at amino acid 858 in exon 21. Treatments for these mutations are highly effective. The mutation in exon 19 is noted in 48.2% of patients and the mutation in exon 21 is noted in 42.7%. The figure on the right shows therapeutic effectiveness, which ranges from 70 to 80%. These treatments clearly have a higher level of antitumor action than treatments for a mutation in exon 18 or exon 20.

Slide 18-19

If a patient is positive for a mutation in the EGFR gene, why is treatment effective? This is a crucial clinical issue. EGF (a ligand) binds to EGFR (a receptor), and that stimulus is conveyed to a tyrosine kinase domain in cells. TP binds to the ATP-binding pocket of the tyrosine kinase domain, so the signal is transmitted to the nucleus of cells and cancer grows.

Slide 20-22

As I explained a moment ago, a ligand binds to a tyrosine kinase receptor. As shown in the slide, a dimer is formed and ATP binds to the ATP-binding pocket of the intracellular kinase domain. When, however, a mutation occurs in the EGFR gene, the structure of the ATP-binding pocket changes, and an EGFR-TKI readily binds to it. ATP is precluded from binding, and phosphorylation does not occur. The signal is not transmitted to the nucleus, and the growth of cancer is inhibited.

Slide 23

This slide shows a patient with EGFR mutation-positive adenocarcinoma of the lung who was treated with gefitinib (an EGFR-TKI). As a result of treatment, the primary foci and brain metastases indicated by the white arrows shrank markedly. A drug has difficulty reaching the brain because of the brain-blood barrier (BBB), but the drug proved to be efficacious even against brain metastases.