Hokushin e-learning for cancer specialists
Genomic Medicine
The Basics of Genomic Medicine and Cancer Genomes
Cancer Epigenomes

Takeshi Suzuki (The Cancer Research Institute of Kanazawa University)

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This e-learning lecture on the Basics of Genomic Medicine and Cancer Genomes, Chapter 4, Cancer Epigenomes will now begin.
I am Takeshi Suzuki of the Cancer Research Institute of Kanazawa University and I will be teaching this chapter.

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Based on what you have learned thus far, you now understand that cancer is a genetic illness. Cancer develops as a result of gene mutations and abnormal gene expression, so genomic changes, i.e. changes in genetic information, are crucial. Genomic changes are irreversible and are consistently passed on to the next generation of cells via cell division. In the past, cancer was thought to develop primarily due to genomic changes. However, genomic changes are typically infrequent, and those changes alone are unlikely to cause cancer to directly develop. What explains this are epigenomic changes. With epigenomic changes, the pattern of expression changes but genetic information itself does not change. Epigenomic changes are reversible. Thus, one could envision a treatment strategy to return the epigenome to its normal state, so these changes have garnered attention as a target for the next generation of cancer therapies. Just what is an epigenome? An epigenome is a concept referring to epigenetic information as a whole. I will start by explaining what epigenetics is.

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Epigenetics is the mechanism causing phenotypic changes without causing changes in gene sequences. Epigenetics is mechanism to decipher the same genomic information in various ways. The human genome codes for around 22,000 genes. However, there are around 200 types of cells, such as neurons and muscle cells, in the human body. Every cell has the same genome and the same genetic information, but why are multiple cells with completely different phenotypes produced? The answer is the particular manner in which genes are expressed in each of those

cells. The mechanism that determines ow genes are expressed is epigenetics.

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So what sort of mechanism is epigenetics? What exactly is an epigenome? Do cells contain epigenomic information? If so, in what state? Those questions must be answered. There are 2 simple answers, posttranslational modification of histones and methylation (modification) of genomic DNA. Histones are basic proteins that comprise nucleosomes, which are the basic units of chromatin. Histones bind to DNA and store DNA in the nucleus of a cell. These histone proteins are chemically modified, e.g. they are methylated or acetylated. Modification of histones and methylation of DNA can be reversed or counteracted, but those changes can be consistently passed on to new cells after cell division.

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I will now explain about histone proteins in detail. The human genome contains around 6.4 billion base pairs. DNA has a total length of about 2 meters. DNA is stored in the nucleus, which has a diameter of 5 to 10 micrometers. What makes that possible is a structure known as chromatin. Chromatin is primarily a complex of DNA and histone proteins. The basic units of chromatin are nucleosomes. The 4 histones H2A, H2B, H3, and H4 are known as core histones, and an octamer or histone octamer contains 2 copies of each of the 4 core histones. A nucleosome is DNA wrapped around an octamer. Chromatin produces histones, and the structure of chromatin is vital to every event involving DNA, including control of transcription and DNA replication and repair. Epigenetics can be referred to as chromatin biology. As an example, chromatin exists in a less condensed form as euchromatin in a genomic region with abundant gene expression while it exists in a condensed form as heterochromatin in a region where gene expression is quiescent.

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Once histones are synthesized, they undergo various types of chemical modifications. These modifications include acetylation, methylation, phosphorylation, and ubiquitination. Certain amino acid residues are chemically modified, and the main residues are listed here for each histone. Lysine residues and arginine residues undergo acetylation and methylation, serine residues and threonine residues undergo phosphorylation, and lysine residues undergo ubiquitination. Research on the relationship between posttranslational modification of the histone H3 and its function has proceeded. This has revealed that the amino terminus referred to as the tail undergoes various modifications, resulting in a variation of functions.

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Epigenetic marks such as histone modification are consistently passed on to future generations of cells, but they change dynamically in accordance with the stage of development and environment. まず, Writers are induced by guides called initiators, which are molecules known as long-chain non-coding RNAs, to leave a mark at a specified site. Readers are molecules that interpret those marks, which erasers are molecules that erase those marks. Remodelers are molecules that alter the position of nucleosomes and the structure of chromatin, and insulators are molecules that form boundaries limiting the extent of epigenetic control. Dynamic epigenetic control and control of chromatin function are achieved these molecules fulfilling their various roles.

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The most well-known type of control is the histone code. The histone code refers to the hypothesis that a combination of chemical modifications to histones induces chromatin-specific functioning. As an example, there are lysine residues at 4 locations at the tail end of the amino terminus of histone H3 that mainly undergo methylation (modification). There are enzymes that methylate individual lysine residues and demethylating enzymes that remove methyl groups. Lysine 4 is referred to as K4 and lysine 36 is referred to as K36. Methylation of K4 and L36 is related to the activation of transcription, while methylation of K9 and K27 is linked to the suppression of gene expression.

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These enzymes and proteins are the writers, erasers, and readers involved in the methylation of the histone H3K4. Writers are histone lysine methyltransferases (KMTs); these enzymes add a methyl group to K4. Erasers are histone lysine demethylases (KDMs). Readers include proteins with a PHD domain that recognize methylation sites, and readers serve as an effector of methylation (modification) signaling. Methylation of K4 is related to the activation of transcription.

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Next are proteins involved in the methylation of K27. A well-known writer is the enzyme EZH2. EZH2 is an important enzyme that is closely associated with the development and malignant transformation of cancer. Enzymes that typically control histone modification form a protein complex consisting of multiple proteins. EZH2 is a subunit of polycomb repressive complex 2 (PRC2) that catalyzes the methylation of K27. K27 erasers include demethylating enzymes such

as UTX. A well-known reader is the CBX7 protein, which is a component of polycomb repressive complex 1 (PRC1). The CBX7 protein recognizes and binds to K27 methylated by PRC2 and it monoubiquitinates the core histone H2A, thus inducing the suppression of gene expression.

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The concept of the histone code was accepted about 10 years ago. Since then, a succession of studies has reported abnormalities in the methylation (modification) of histones and abnormalities in the enzymes regulating methylation in cancer. To summarize the major finding of those studies, abnormalities in most of the enzymes that methylate and demethylate the histone H3 have been detected in cancer. Most abnormalities related to gain of function (GOF) involve high levels of expression of those enzymes in cancer while abnormalities related to loss of function (LOF) involve a mutation in or suppressed expression of those enzymes. An interesting fact is that the same enzyme, e.g. the enzyme EZH2, can have GOF or LOF depending on the type of cancer. That is, a mutation linked to the development of cancer can act in the exact opposite manner as a result of differences in the cell context. Enzymes control which genes to target, but they can act differently depending on the cell type and conditions.

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Next, we will look at abnormalities in the regulation of H3K27 methylation in detail. EZH2 is an enzyme that methylates K27, and it is a catalytic subunit of the complex PRC2. EZH2 is an oncogene. This is because amplification of the EZH2 gene reveals high levels of its expression in many solid tumors and because mir101, a microRNA that suppresses EZH2 expression, is detected in cancer. Moreover, a GOF variant of EZH2 that has increased enzymatic activity as a result of a point mutation is detected in leukemia, and an LOG variant of UTX, an enzyme that demethylates K27, is detected in numerous cancers. This is evidence that increased methylation of K27 is associated with cancer. In contrast, an LOF variant of EZH2 has been identified in some leukemias and lymphoid tumors, suggesting that EZH2 also acts as a tumor suppressor gene. That is, EZH2 can act as an oncogene or as a tumor suppressor gene depending on differences in the cell context. Thus, abnormal K27 methylation in cancer is not a simple issue. Instead, caution is required when formulating a treatment strategy to target that abnormality.

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Next, I will explain about the acetylation (modification) of histones and their functions. These

proteins are involved in the acetylation of histones. Writers are histone acetyltransferases (HATs), and the protein CBP is a well-known writer. Erasers are histone deacetylases (HDACs), and there are numerous types. Readers include proteins with a bromodomain that recognize acetylation sites, and readers facilitate acetylation signaling. One characteristic that differentiates methylation from acetylation is that both groups of enzymes involved in the acetylation of histones do not specifically act on amino acid residues at certain sites. Using the histone H3 as an example, lysines such as K9 and K27 are acetylated, but certain enzymes are not restricted to acting only on certain sites. Another characteristic is that acetylation is basically related to the activation of transcription, regardless of the site that is acetylated. This differs completely from control via methylation.

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So how is the acetylation of histones controlled in cancer? The mechanism is simpler than that for methylation. Mutations and fusion genes are detected in cancer and produce LOF variants of writers, i.e. acetylating enzymes. In contrast, there are few mutations in erasers. An abnormality occurs when these enzymes are overexpressed or they are mistakenly transported to a gene other than the original target gene due to a mutation in a transcription factor. Regardless of the change, the abnormality ultimately decreases acetylation, causing the suppression of gene expression. The relationship between that phenomenon and cancer is being discussed.

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Next, I will talk about the methylation (modification) of DNA separate from histones. DNA methylation refers to substitution of a methyl group for the hydrogen bound to the fifth carbon of cytosine, which is one of the bases that make up DNA. The enzymes responsible for this reaction are known as DNMTs. A reaction to remove a methyl group is known as demethylation. Until recently, active DNA demethylation pathways as shown here were unclear. Previously, a reaction known as passive demethylation was considered to be the only pathway for DNA demethylation. In other words, DNA was replicated and cytosine was taken up by the newly synthesized DNA strand without being methylated. If replication was repeated while cytosine was still unmethylated, demethylation of DNA would occur spontaneously. As was recently revealed, what holds the key to the active demethylation of DNA is an enzyme known as TET that controls the initial stage of the reaction.

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DNMTs are writers of DNA methylation and can be divided into 2 groups based on their action.

The first is DNMT1, which is a maintenance methylase. During DNA replication, unmethylated cytosine is taken up by the complementary strand and the cytosine site is partially methylated. The maintenance methylase known as DNMT1 methylates the complementary strand. The second group of DNMTs includes DNMT3A and DNMT3B, which are de novo methylases that attach a methyl group to unmethylated DNA. The TET family of enzymes, which are involved in active demethylation, are important erasers. Readers are well-known proteins, such as MBDs, that can recognize and bind to methylated cytosine and that are involved in the regulation of gene expression.

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An important aspect of the regulation of gene expression via DNA methylation is the concept of CpG islands. A CpG island is a place where CG dinucleotides often appear in a genome. There are CpG islands in promoter regions and expression-regulating regions of about half of human genes. When a CpG island promoter is not normally methylated, a gene is constantly expressed. When, in contrast, a CpG island promoter is highly methylated, gene expression is consistently suppressed.

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The abnormal methylation of genomic DNA has long been studied in cancer. It has 2 characteristics. One is that a decrease in the methylation of DNA occurs in a wide range of genomic regions. The other is that extensive methylation is noted in a local CpG island promoter. In normal cells, for example, heterochromatin regions near the centromere at the center of a chromosome are hypermethylated, and gene expression is constantly turned OFF. In cancer cells, hypomethylation of DNA occurs genome-wide. Repeats have an open chromatin structure, and transposons not expressed in normal cells are expressed. The frequency of recombination and genomic instability increases. In normal cells, genes are expressed while the CpG island promoter is hypomethylated, but local hypermethylation occurs in cancer cells, and gene expression is constantly turned OFF. When this occurs due to a tumor suppressor gene, the tumor suppressor gene is silenced, leading to oncogenesis.

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Next, we will look at the relationship between the TET protein and cancer in relation to DNA demethylation. An LOF variant of the TET2 enzyme is often detected in leukemia and glioblastoma. In these cancer cells, hypermethylation of CpG islands is induced due to diminished demethylation. In the same types of cancer, mutations in the isocitrate

dehydrogenase enzymes (IDH1 and IDH2), which are involved in glycolysis and the TCA cycle, are also detected. These enzymes convert isocitrate to α-ketoglutarate, but the enzyme variant produces a metabolite known as 2-hydroxyglutarate (2HG). 2HG competitively inhibits the activity of the TET family of enzymes (which are α-ketoglutarate-dependent) and histone-demethylating enzymes with the Jumonji domain and subsequently induces CpG island hypermethylation. An LOF variant of TET2 and an IDH variant are mutually exclusive in cancer. In other words, hypermethylation of CpG islands is crucial to the development of cancer. Incidentally, a substance like 2HG, i.e. a metabolite associated with cancer, is referred to as an oncometabolite.

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Well, I have explained about epigenomic changes in cancer. As I initially mentioned, epigenomic changes are reversible, so one could envision a treatment to return the epigenome to its normal state. Here, I will describe well-known examples of treatments targeting the epigenome. One treatment involves azacitidine and decitabine, which are DNA methylase inhibitors. These compounds inhibit every form of DNMT3. When used at a low concentration, they are efficacious in treating MDS myelodysplastic syndrome, so they have been approved by the FDA. Another treatment involves the histone deacetylase (HDAC) inhibitor vorinostat. This drug is approved for treatment of T cell lymphoma. These drugs halt the silencing of tumor suppressor genes by reducing the methylation of DNA or increasing the acetylation of histones. This action inhibits the proliferation of cancer cells. Epigenomic drug discovery has garnered attention as other compounds targeting the methylation of histones are being successively developed.

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This slide summarizes the content of this lecture. In cancer, genomes and epigenomes influence one another. Epigenome regulators are affected by mutations in the genome. Subsequent epigenomic changes pave the way for genomic changes. Mutations or instability in the genome and epigenomic abnormalities act in a coordinated manner and are involved in determining various phenotypes associated with cancer and characteristics of cancer cells を involved in determining, as I hope you now understand.

With that, I'd like to conclude my lecture on cancer epigenomes.