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Need for Epigenetic-Based Cancer Research — Cancer Research in the Post-Genome Era

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2.1 Introduction

With analysis of the human genome complete (actually, 98.8%), the Human Genome Project was declared complete on April 14, 2003, heralding the arrival of a genuine post-genome era.

In fact for about five years now, research strategies have been targeted to suit this post-genome era. In Japan, genome-based research has also been promoted such as that on protein structure/function analysis, sugar-chain structure/function analysis and disease-related SNP analysis as part of post genome research.

In this context, research focusing on the causal relationship between diseases and the human genome has become internationally widespread. In recent years, it has been reported that histone protein (which binds to genome DNA) or genome DNA in the cells from patients with various types of cancers and with common diseases might be modified by enzymes such as methylase (methylation) or acetylase (acetylation). This type of modification is considered to affect gene transcription and expression, arousing interest among researchers in this field as possible causes of diseases[1]. The modification is a reversible reaction catalyzed by biological enzymes and its rate of occurrence depends on external factors including exposure to chemicals consumed in food or present in the environment.

Referred to as "epigenetic," this modification is an essential mechanism for normal cells to sustain life. Therefore, an abnormality within this mechanism may result in diseases including cancer.

This report provides descriptions of "epigenetics" as a likely area of cancer research, including an introduction to epigenetics, its causal relationship with cancer and the current international trend in epigenetic-based cancer research.

2.2 What does epigenetic mean?

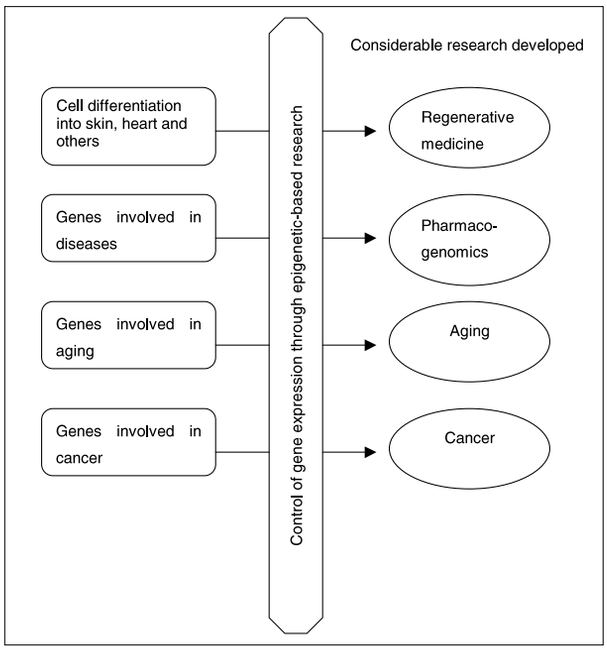
The word "epigenetic" is derived from epigenesis as opposed to ontogenetic preformation, a philosophy that prevailed in the biological field in the 17th-18th centuries. According to preformation, an individual living organism is established through a course of development from the potential properties it has inherently, whereas in epigenesis, the living organism undergoes internal and external effects sequentially in the process of ontogenesis, ultimately evolving into an individual.

At this time, "epigenetic" is a phenomenon that affects gene expression by a mechanism other than genomic mutation. Epigenetic-based research is expected to reveal the mechanism for suppression of gene expression, facilitating progress in the research on gene expression in various interdisciplinary fields (Figure 1).

2.2.1 Epigenetics in normal living organisms

Epigenetics play an important role in sustaining normal living organisms. The genome DNAs, which are extracted from the cells of the skin, heart or liver of a human body, are basically the same. Contrary to the description above, it has been reported that each gene in these cells has different expression patterns. This means that only genes associated with the formation of the skin, heart or liver are expressed. This type of suppression of gene expression is achieved through epigenetics, which develop in the process of cell differentiation after the genome

Figure 1: Considerable research areas developed by epigenetic-based research



is determined. In other words, gene expressions of all the cells in the living organism (excluding germ cells) are controlled by epigenesis.

Epigenetic suppression of gene expression in normal somatic cells has been shown to inactivate the X-chromosome and genomic imprinting in the cells[2].

One of two X-chromosomes in a female cell, one inherited from the father and another from the mother, is inactivated. This means that usually, a female's cells have mosaic patterns in which "inactivated cells with the X-chromosome inherited from the father" and "deactivated cells with the X-chromosome from the mother" are randomly mixed. Daughter cells inherit X-chromosomes deactivated at the early stage of embryonic growth through cell division. Inactivation of X-chromosomes in female cells compensates for the imbalance in the number of genes between the male, who has one X-chromosome and the female, who has two X-chromosomes.

A human has 23 chromosomes each inherited from both his/her mother and father, totaling 46 in one normal cell. The chromosome consists of a DNA-protein complex (chromatin). Therefore, there are two sets of genes in the cell, one is from the father and the other is from the mother. Gene imprinting is when the genes from the mother's and father's families express differently. The suppressed gene is referred to as an imprinted gene.

It has been reported that either of the two epigenetic mechanisms mentioned above occurs at an early stage of embryonic growth and differentiation. However, the details of their mechanism and significance to the living organism have not yet been revealed.

2.2.2 Epigenetic mechanism for suppression of gene expression

The epigenetic mechanisms for suppression of gene expression include DNA methylation, histone acetylation/methylation and chromatin remodeling (Table 1).

These epigenetic mechanisms are caused by modifications of enzymes in DNA or protein. Techniques such as the Sanger method could not detect the modification, because the modification is lost during the chemical treatment used for detection. Since no modification could be detected in a trace amount of sample, fewer studies have been reported on the causal relationship between diseases and epigenetics. The bi-sulfite sequence method developed in 1992 enabled detection of DNA methylated patterns in a trace amount of DNA sample. In addition, the DMH (differential methylation

Table 1: Epigenetic mechanism for suppression of gene expression

Mechanism	Action
DNA methylation	It causes methylation of cytosine in the CpG sequence of genome DNA, inhibiting gene transcription or gene expression by disturbing access of transcription factors and some enzymes to the DNA.
Histone, acetylation and methylation	It causes acetylation or methylation in histone, changing the structure of a DNA complex, histone and others, and affecting gene transcription and expression.
Chromatin remodeling	It causes the chromatin structure to condense or relax, inhibiting or facilitating gene transcription or gene expression by controlling access of transcription factors and some enzymes in the chromatin (which is composed of DNA, histone and others).

hybridization) method using micro arrays developed in 2000 and the MethyLight method, a real time PCR method using fluorescent dyes reported in 2001, dramatically improve the DNA methylation detection accuracy and rate[3,4].

These innovative detection techniques have enabled research on the causal relationship between diseases such as cancer and DNA methylation. Therefore, researchers specializing in cancers have recently focused attention on DNA methylation. In the following paragraphs of this report, DNA methylation is highlighted and discussed.

2.2.3 Mechanism for suppression of gene expression by DNA methylation

In the process of DNA methylation, a methyl group binds to the 5th position of the cytosine base in the DNA molecule by DNA methyltransferase, producing 5-methylcytosine. Since methylation at site 5 does not affect base paring, 5-methylcytosine can pair with a guanine base. This means that DNA can be replicated normally. However, the structural difference of 5-methylcytosine can affect gene expression.

Generally, gene expression requires gene regulatory protein and transcription factor to bind to the promoter region of the gene (the DNA sequence of the promoter region involved in gene transcription). During gene expression, the gene regulatory protein and the transcription factor bind to the promoter region, which prevents DNA methyltransferase structurally from approaching the DNA sequence in the promoter region. At this stage, DNA methylation cannot occur.

When most of the gene regulatory protein and the transcription factor are released from the promoter region, DNA methyltransferase can approach the DNA in the region. Accordingly, the DNA in the promoter region is readily methylated. The DNA, once methylated, is specifically bound by internal protein to prevent the DNA from being demethylated. This means that DNA methylation is maintained, with gene expression stably inhibited.

In addition, the methylation pattern occurring in a parent DNA chain is readily inherited by its daughter DNA chain through maintenance methylase. In other words, when the DNA is methylated in the genome in a cell, all its daughter cells will have the methylated DNA at the same site as those of their parent genome.

An especially important mechanism is that which occurs in the course of cell differentiation, for example, preventing skin cells from transforming into heart, liver cells or any other cells except skin cells after cell differentiation.

2.2.4 DNA methylation in normal living organisms and that involved in diseases

DNA methylation inhibiting the expression of unnecessary genes in the living organism at the normal stage has been described. This suggests that DNA methylation is involved in diseases through the following: (i) DNA methylation occurring in any way at a different region from the normal region, inhibits the expression of essential genes for sustaining a living organism, or (ii) although the DNAs have been stably methylated in a normal region, when the same region is demethylated, this facilitates the expression of unnecessary genes, which disturb the development of a normal living organism.

How this relates to cancer caused by DNA methylation will be discussed in Chapter 2.4.

2.3 Scientific publications in the field of epigenetic-based research

To analyze the trend in epigenetic-based research, the number of scientific articles published (from 1993 to 2002) on epigenetic-based research was investigated using the keyword search function of PubMed, a database containing medical articles (Figure 2).

The result of the search for reports using the keyword “epigenetic” showed that the number of reports relatively increased in 1996 and 1998 compared with those in the previous years, while it indicated a remarkable increase in 2000 and later. The search by the keywords “epigenetic and cancer” or “methylation and cancer” also showed almost the same results as those mentioned above.

In recent years, it has been reported that epigenetics might be involved in aging and diseases other than cancers, suggesting that the rapid increase in the number of articles reflects

the results of these studies. Specifically, this is the reason why the number of articles on epigenetic-based research drastically increased in 2000. At that time, as the Human Genome Project was approaching its end, epigenetic-based research began to attract greater attention as a post-genome target, and the useful techniques for detecting DNA methylation mentioned before were identified.

The increase in the number of articles mentioned above suggests that epigenetic research and epigenetic-based cancer research are rapidly growing new research fields.

2.4 Epigenetics can be linked to cancer

Progress in molecular biological research has contributed to the great dissemination of the realization that cancer may be caused by any anomaly in the genome or genes. In recent years, epigenetic-based research has attracted worldwide attention because it has been revealed that epigenetics may cause cancer[5].

2.4.1 What is cancer?

Cancer can be defined as a disease where: (i) cells proliferate uncontrollably , and (ii) the proliferated cells then invade a region generally occupied by other cells, and remain there (Molecular Biology of THE CELL, 3rd edition). When cancer cells having the characteristic defined in (i) remain in a fixed region, this is referred to as a benign tumor. Usually, a benign tumor can be completely cured by excising the affected area. However, it is difficult to treat cancer referred to as a malignant tumor because it invades neighboring tissues or metastasizes to other organs.

Recent progress in molecular biological research has widely disseminated the realization that the cause for inducing the behavior of cancer cells described in (i) and (ii), is the abnormality in the gene involved in genes such as the "oncogene" or the "tumor suppressor gene." It has been revealed that abnormal genes can largely be grouped into two types, qualitative and quantitative abnormality.

2.4.2 Cancer development by qualitative and quantitative abnormality

Cancer development caused by qualitative abnormal genes has attracted the greatest attention because many abnormal genes (mutant genes) are found in cancer cells, and these are involved closely in cancer development as well as the malignant differentiation of cancer.

In the course of cancer development due to quantitative abnormal genes in cancer cells at the gene expression level, the normal cell cycle

Figure 2: Scientific publications in the field of epigenetics (1993-2002)

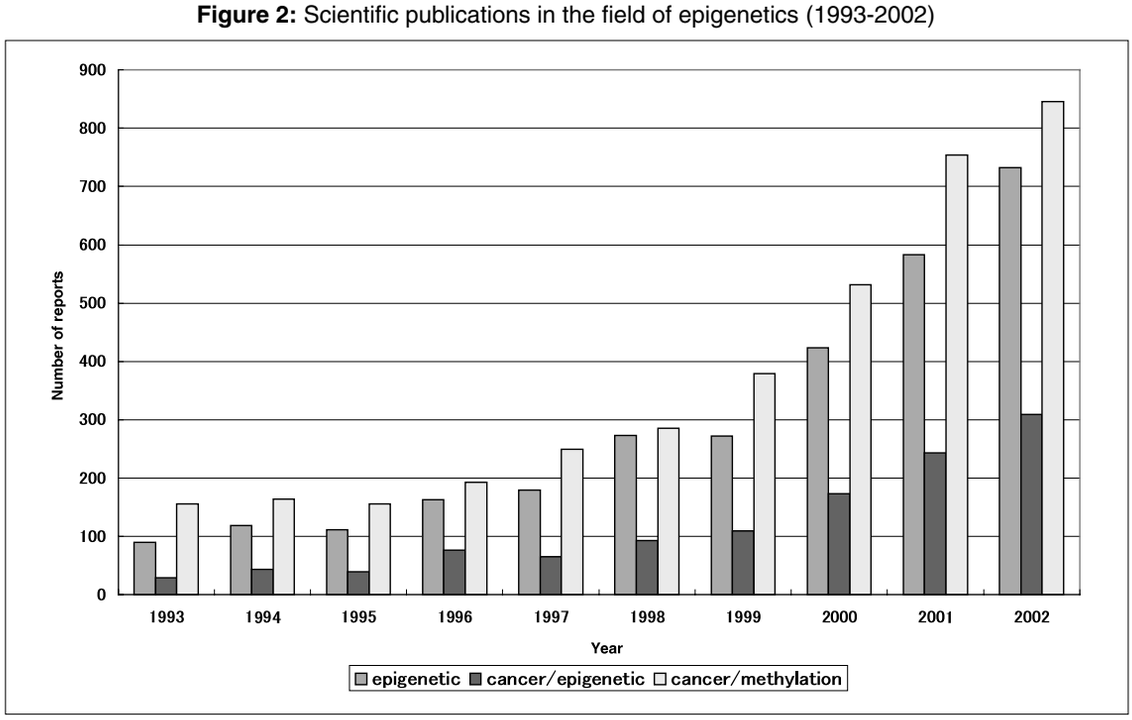


Table 2: Methylated genes observed in cancer cells

Gene	Gene function	Cancer type
P14 (ARF)	cell cycle	colon and rectum cancer, stomach cancer
P16 (INK4a)	cell cycle	lymphoma, pancreatic cancer, colon and rectum cancer, stomach cancer
hMLH1	DNA repair	colon and rectum cancer, cervical cancer
BRCA1	DNA repair, cancer suppressor	ovarian cancer, breast cancer
MGMT	DNA repair	colon and rectum cancer, brain tumor
GSTP1	neutralization of toxicity	liver cancer
DAPK	apoptosis	lymphoma
CDH1	cell adhesion	esophageal cancer
TIMP3	proteolysis inhibitor	kidney cancer
APC	tumor suppressor	stomach cancer, pancreatic cancer, liver cancer

Source: Author's compilation based on a reference[6]

cannot be maintained. This is because redsuced or suppressed transcription activities of the tumor suppressor and DNA repair genes may lead to a decrease in proteins. An enhancement in the transcription activity level may cause cancer by increasing the amounts of oncogene and oncoprotein, disabling control of the cell cycle. This suggests that in addition to abnormalities in the genomic DNA itself, epigenetic factors can also be involved in cancer development.

2.4.3 Methylation of DNA in cancer cells

Methylated genome DNAs are frequently observed in a cancer cell. In recent years, the causal relationship between DNA methylation and cancers has been revealed.

Table 2 shows cancer types and the methylated genes. It has been reported that methylated genes have been observed in various types of genes associated with every stage of the cell cycle including the tumor suppressor gene[6]. While some genes are commonly methylated in many types of cancers, other genes are methylated in specific types of cancers. This suggests the existence of different causal mechanisms for cancer depending on cancer type.

Collecting and collating information on methylated genes to be integrated into a database for use as fundamental information may be a requirement for facilitating future research on cancer toward treatment.

2.4.4 The pattern of methylated DNA in cancer cells

Many patterns of methylated DNA patterns are observed in a cancer cell[1] including a wide range of hypomethylation/demethylation[2] and site-specific (ex., a gene promoter region) hypermethylation[7,8].

Hypomethylation or demethylation has been reported to disable control of the expression of unnecessary genes in the cells which form each tissue or organ (for example, all the genes expressed in liver cells are not necessary for the gene expression of skin cells). This might lead to inducing instability in chromosomes and increasing the risk of abnormal development of cells. Hypomethylation is observed over a wide range of genes in many cases.

In site-specific hypermethylation, methylation has been concentrated in the specific region related to gene expression, that is, the gene promoter region. This leads to inhibition of expression of the tumor suppressor gene and the DNA repair gene, which play roles in the suppression of tumors or cancer development.

Table 3 shows differences in the patterns of methylated genes between normal and cancer cells.

2.4.5 Substances possibly involved in DNA methylation

Recently, it has been shown that deficient or excessive intake of some substances contained in food might affect the methylation of normal cells.

Table 4 shows the substances, which have been reported to affect DNA methylation. Although details on the mechanism have not been revealed, it is suggested that when deficiency exists in any substance with a chemical structure that can provide a methyl group, hypomethylation (instable chromosomes) is likely to be induced in the DNA of cells[9-12].

It has been reported that ingesting particular foods (not containing choline and folic acid) containing an environmental pollutant such as arsenic may accelerate DNA hypomethylation in animal experiments. This means that further investigation is required to establish safety standards for arsenic.

2.4.6 Chemical substance deficiency-induced cancers, and cancer prevention by supplementation of deficient substances

The causal relationship between deficiency of chemical substances that can provide a methyl group and cancer development has been studied since the mid-1980s. One report showed that cancer and tumors developed in the livers of experimental animals (rats) at a high rate of incidence by continuous feeding with food containing no choline for one to one and a half years. The DNA methylation patterns of their cells were different from .those of normal cells and exhibited hypomethylation. At that time, since sufficient techniques for detecting DNA methylation were not available, no further detailed research was conducted.

At present, it is believed that the deficiency of chemical substances can be correlated with the increased risk of cancer development. Thus, the target of research has been changed to cancer

Table 3: Methylated genome DNAs observed in normal and cancer cells

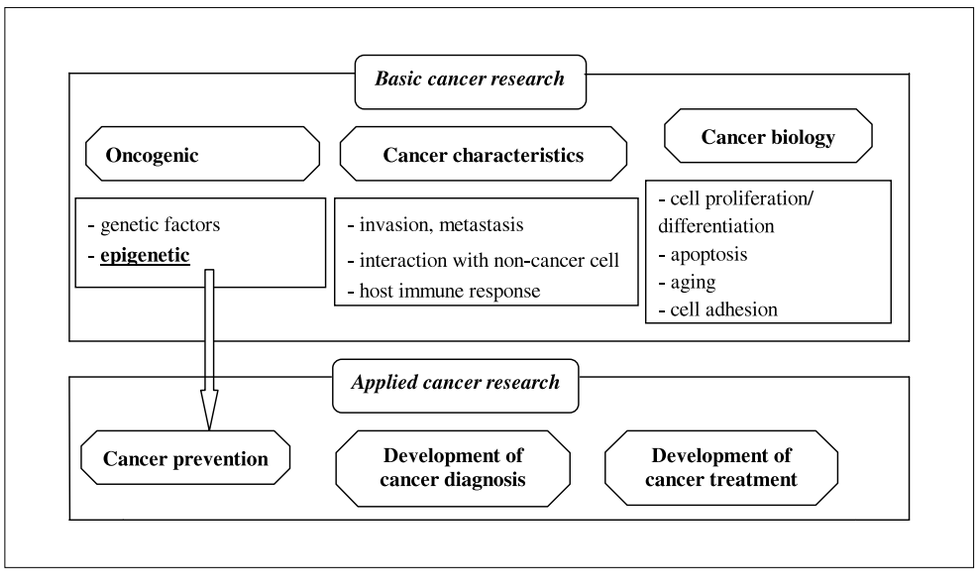
Example of genome DNA sequence	Normal cell	Cancer cell
Repetitive DNA sequence with the same pattern such as CTG	methylation	demethylation (hypomethylation)
Viral sequences in human genome DNA	methylation	demethylation (hypomethylation)
Promoter region of tumor suppressor gene	demethylation (hypomethylation) methylation	methylation

Table 4: Substances possibly involved in methylation

Substance	Food containing the substance	Intake	Methylation type	Gene state
methionine	animal protein	deficiency	hypomethylation	unstable
Choline	yolk	deficiency	hypomethylation	unstable
folic acid	green and yellow vegetables	deficiency	hypomethylation	unstable
folic acid	green and yellow vegetables	deficiency	methylation	inhibits tumor suppressor gene p53
Vitamin B_{12}	egg, seafood	deficiency	hypomethylation	unstable
Zinc	shellfish	deficiency	hypomethylation	unstable
selenium	mushroom, sea weed	deficiency	hypomethylation	unstable
Retinoic acid	eel, liver, yolk	excess	hypomethylation	unstable
alcohol	alcoholic beverages	excess	hypomethylation	unstable
Arsenic	environmental pollution (contaminated drinking water)	excess?	hypomethylation	unstable

Source: Author's compilation with reference to NCI HP and references[9-12]

Figure 3: Cancer research map



prevention by the supplementation of deficient chemical substances.

In 2000, a study was conducted on the effect of folic acid contained in food (fed for 1-2 months) on experimental animals (mice), whose genes were altered so that carcinoma of the colon and rectum could be induced[13]. In the group (folic acid group) to which food containing folic acid was given, the normal state of methylation was observed compared with the non folic acid group. In the group to which the food containing folic acid was continuously given prior to the development of intestinal tumors, a lower rate of polyp incidence was observed compared with the non folic acid group. However, giving food containing folic acid after tumor formation had no effect. This suggests that folic acid should be supplied at a proper timing to prevent cancer development.

2.5 Worldwide trend in epigenetic-based cancer research

External factors including exposure to chemical substances in the environment, food and lifestyle may increase the risk of cancer development. To reduce the risk of cancer development, external factors, which may induce cancer development, should be eliminated or controlled. This may be achieved by applying the results of epigenetic-based research.

As shown in Figure 3, epigenetic-based cancer research falls within the category of "studies on the elucidation of the oncogenic mechanism." The results of this research may directly contribute to the research on cancer prevention.

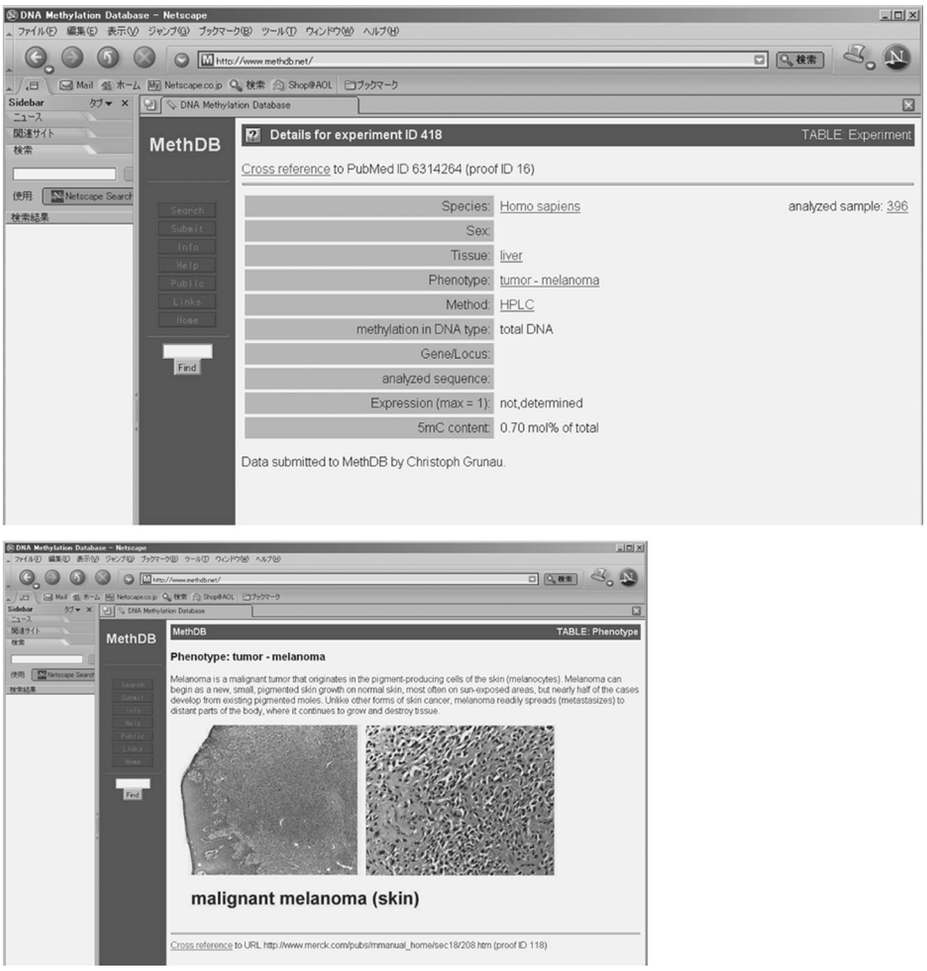
International epigenetic-based research has been progressing as part of the Post Genome Project for about five years in collaboration with EU members. In the U.S., a research project has also been launched.

Regrettably, in Japan, no international projects on epigenetic-based cancer research or research projects in collaboration with multiple domestic institutes have been launched yet.

2.5.1 Trend in epigenetic-based research in the U.S.

In the U.S., although no large-scale projects on epigenetic-based research have been launched, various workshops and conventions focusing on the 'epigenetic' theme have been held: "Diet, DNA methylation processes and health" supported by the NIH, FDA and the U.S. Nutritional Science Society in August 2001, "Epigenetics in Cancer Prevention: Early detection and risk assessment"at which researchers from the Cancer Prevention Department, NCI, attended as the main members in December 2001, and "Cancer genetics & epigenetics" as part of the Gordon Conference in January 2003, in which studies on the causal relationship between epigenetic phenomena such as methylation and cancer development were presented and the possibility for further study

Figure 4: DNA Methylation DB (Example: Transfer of malignant melanoma to liver)



Source: HP of DNA Methylation DB

was discussed. Additionally, in September 2002, the call for research on a new theme, "Diet, DNA methylation and other epigenetic events, and cancer prevention," was announced. Researchers can apply for an NIH Research Grant (R01 or R21) for this. The NCI will allocate about 2.5 million dollars (3 million yen) to grants from the fiscal year 2004 budget.

2.5.2 Epigenetic-based research strategy in Europe (Human Epigenome Project)

Europe has launched an epigenetic-based research project ahead of the U.S. and Japan. Aiming at the elucidation of epigenetic information in the human genome, the Human Epigenome Consortium was formed in 1999. The Sanger Center (UK), Centre National de Genotypage (France), German Cancer Research Center, Berlin Institute of Technology, and Max Planck Institute of Molecular Genetics (Germany) have participated in the consortium as members.

In the first stage, the Human Epigenome Project focused on making a detailed methylated gene map. The map will be useful for further studies on epigenetics.

Epigenomics, a biotechnology company based in Berlin (Germany), has also been participating in the project as a team member. Epigenomics (http//www.epigenomics.com) aims at the development of epoch-making technologies using the human genome including that of drugs tailored to individuals. Through joint research, the company has formed an international business network and, furthermore, strengthened its business network in the U.S. by operating a branch in Seattle.

2.5.3 Building of a DNA Methylation Database in Germany

Germany has built the world's first DNA methylation database[14,15]. Called MethDB, this is the first methylation database accessible to the

public(http//www.methdb.net). As of September 4 in 2002, 6,667 methylation data on 46 kinds of species, 160 kinds of tissues and 72 kinds of expression phenogenetics are contained in the database. The number of accesses to the database per month has increased on a monthly basis from 810 in June 2000 to 8,884 in July 2002. This database has incorporated various innovative ideas. One of them, stoichiological image data, in which gene expression phenogenetics is represented in a micro graphical format, is available to users (Figure 4).

This database contains a wide range of data on DNA methylation in cancer cells. It is expected to play an important role as a source of information for future epigenetic-based cancer research.

2.6 Conclusion

Although epigenetic-based research is still at the development stage, this area will undoubtedly progress rapidly and enlarge to eventually become an international trend. Therefore, Japan needs to refine its domestic research system for epigenetics, while paying strong attention to the current movement of international epigenetic research.

Listed below are the points to be considered when discussing the refinement of a system for future epigenetic-based cancer research under the leadership of the Japanese government.

(1) Enlargement of future epigenetic-based cancer research.

An interdisciplinary research group (across the fields of medical science, natural and physical science, food, nutrition, health, and immunology) should be formed for the epigenetic research project.

(2) Building of a domestic human epigenetic database.

Data on epigenetic research such as DNA methylation, conducted at each institute and university, should be integrated into the main database to allow researchers to use and share the data for disease prevention and diagnosis studies. The database can be controlled by a satellite system, meaning that the database is located at each institute and university, while the main database can be located in cyber-space.

(3) Giving epigenetic-based research the status of post-genome research.

In Japan, post-genome research has focused on the protein structure/function analysis and SNPs. Epigenetic-based research must also be positioned as a pillar of post genome research.

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