Present State of Gene Diagnosis and Future Prospects

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Abstract: The entire base sequence of the human genome has almost been clarified. In the post genome era of the 21st century, both genetic alterations and epigenetic abnormalities, especially abnormalities in the gene expression caused by methylation of DNA or acetylation of histones can be analyzed easily and quickly with new technology including DNA chip, RNA chip and protein chip. Therefore it is necessary to establish the TNM-G classification with criteria both for genetic abnormalities associated with cancer and precancerous lesions and epigenetic abnormalities. According to the new “TNM-G classification” it is possible to achieve the following: more precise diagnosis; precognition of malignancy, metastasis and recurrence; diagnosis of susceptibility to drugs or cancer and development of new therapies. When a patient with cancer receives the most suitable treatment and prevention based on the characteristics of the patient, the mortality and morbidity rates in this country will decrease by around 2030. In the case of gene diagnosis, the protection of private information at the ethics level and cancer notification must be kept in mind. It is expected that common guidelines on genetic analysis will be drawn up as soon as possible.

Key words: Gene diagnosis; Characteristic diagnosis of cancer; Hereditary tumor; TNM-G

Introduction

The multistage carcinogenesis and the precancerous lesion in each organ was analyzed extensively at the molecular level over the last decades. The results show that abnormality of structure and function in the oncogene, tumor suppressor gene, DNA repair enzyme gene, and other cancer-related genes causes the multistage process of carcinogenesis.\textsuperscript{1)} The combination and order of various kinds of genetic abnormalities depend on the place of carcinogenesis and tissue-type of cancer.\textsuperscript{2)} Furthermore, susceptibility to carcinogenesis, sensitivity to the anticancer drug, and drug tolerance can be understood beforehand by analyzing the genetic polymorphism.

Currently, genetic alterations relating to the
carcinogenesis and progress of cancer in each organ acts as the most suitable marker for establishing guidelines on the prevention and treatment of cancer. This report describes the genetic abnormalities of the representative cancer and precancerous lesion, as well as the significance and present conditions surrounding gene diagnosis. Finally, we explore future prospects in the area of gene diagnosis and propose a new type of classification in which TNM classification is combined with the gene diagnosis of cancer.

Genetic Abnormalities in Cancer

Cancer is a disease of the gene that develops through a multistage process of abnormality in genes such as the oncogene, tumor suppressor gene, and DNA repair enzyme gene according to genetic and environmental factors. The oncogene can be activated by a single mutation in the allele (point mutation, gene amplification, and DNA rearrangement (chromosomal translocation)), or the tumor suppressor gene can be inactivated by two mutations in the allele (referred to as “two hits” and defined in most cases as a point mutation and deletion).

<table>
<thead>
<tr>
<th>Tumor suppressor gene</th>
<th>Non-hereditary tumor</th>
<th>Hereditary tumor</th>
<th>Gene locus</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rb</td>
<td>Retinoblastoma, lung cancer, breast cancer, osteosarcoma</td>
<td>Familial retinoblastoma</td>
<td>13q14.2</td>
<td>Transcriptional control</td>
</tr>
<tr>
<td>p53</td>
<td>Colorectal cancer, breast cancer, lung cancer, and others</td>
<td>Li-Fraumeni syndrome</td>
<td>17p13.1</td>
<td>Transcriptional control</td>
</tr>
<tr>
<td>WT1</td>
<td>Wilms tumor</td>
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<td>APC</td>
<td>Colorectal cancer, stomach cancer</td>
<td>Familial adenomatous polyposis</td>
<td>5q21</td>
<td>beta-catenin, DLG binding</td>
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<td>p16</td>
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<td>Familial malignant melanoma</td>
<td>9p21</td>
<td>CDK inhibitor</td>
</tr>
<tr>
<td>NF1</td>
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<td>Neurofibromatosis type 1</td>
<td>17q11</td>
<td>Activation of GTPase</td>
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<tr>
<td>NF2</td>
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<td>Neurofibromatosis type 2</td>
<td>22q12</td>
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<td>VHL</td>
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(Quoted from reference)
occurs often in the hereditary tumor. In other words, cancer develops from a mutation that occurs in one allele of the reproductive cell of a parent (that is, the first hit) or in the other allele (the second hit).

Hereditary nonpolyposis colorectal cancer (HNPCC), commonly referred to as the Lynch Syndrome, comprises about 10% of all colorectal cancers and is divided into two types. Lynch type I develops only in the large intestine (mainly right colon) while Lynch type II can also develop in the uterus and ovaries. HNPCC arises from gene instability caused by a reproductive cell mutation of the DNA repair enzyme gene ($hMSH2$, $hMLH1$, etc.). Thus far, identified target genes include the TGF-beta II type receptor gene, $BAX$ gene, $E2F4$ gene, etc. Therefore, it is possible to predict cancer risk and conduct early detection and early treatment by testing for reproductive cell mutation of the tumor suppressor gene and DNA repair enzyme gene.

2. Nonheritable tumors

In most human cancers, the mutations of multiple genes including the oncogene and tumor suppressor gene, induced by carcinogens in the environment such as heterocyclic amines and benzo (alpha) pyrene, and radiation, accumulate in one cell, as described above, leading to cancer development. However, there is an unexpectedly lengthy natural history of about 20 years, that functions as a very important preventive measure to cancer. To stop smoking in the younger generation of 10 to 20-year-olds will likely prevent developing into cancer later.

Cancer development through a multistage carcinogenesis process encompasses the following six biological characteristics: 1. The signal for cell proliferation is continuous. 2. The signal for cell control disappears. 3. Deviate from apoptosis. 4. Maintenance of telomeres or infinite proliferation of cell. 5. Angiogenesis. 6. Invasion or metastatic capability. Cancer metastasis or invasion depends on attainment of the above six characteristics by the cancer cell. The combination of these six characteristics or the order of the carcinogenesis process differs with the place of carcinogenesis and tissue type of cancer. In addition, there are various differentiation levels. Carcinomas and sarcomas differ in gene abnormality. DNA rearrangement (chromosomal translocation) is rarely observed in the carcinoma. On the contrary, there is a known characteristic chromosomal translocation in sarcoma, leukemia, and lymphoma.

The interaction pattern between cancer cell and stroma through growth factor or cytokine also differs significantly depending on the organ.

For example, deletion and mutation of the $PTEN/MMAC1$ gene are observed in 60 percent of malignant gliomas. Furthermore, deletion of the $p16$ gene and amplification of the $CDK4$ and $EGFR$ genes correlate with the progress and malignancy of glioma. In neuroblastoma, one of the representative pediatric tumors, there are clones with different biological properties. In one case, cancer recesses naturally, while in the other case there is a poor prognosis of disease concerning the gene amplification of MYC.

Amplification of the $EGFR$ and $cyclin D1$ genes is reported as a prognostic factor in patients with esophageal cancer, independent from TNM classification. In colorectal cancer, the 18q LOH gene is a separate and important prognostic factor at TNM stage II or TNM stage III. In gastric cancer, gene amplification and excessive expression of the K-sam gene is an important prognostic factor. In lung cancer, usually subject to TNM classification in Japan, the mutation of exon 8 in the $p53$ gene (especially in codon 273 and the H2 alpha helix) is a prognostic factor for patients with non-small cell lung cancer. In prostate cancer, deletion of the 8p21–22 genes and acquisition of the 8q24 gene occurs frequently. Reportedly, increase in MYC copy number and deletion of the 8p22 gene are separate risk factors related to the progress of prostatic cancer at stage III and the cause of death.

The most common genetic abnormality in
human cancer is mutation in the \textit{p53} gene. It is supposed that the tumor cell with a mutated \textit{p53} gene is resistant to apoptosis induced by hypoxia; therefore, cloning of the tumor cell with the mutated \textit{p53} gene is selected for development.

There are various human oncogenic DNA viruses including EBV, HPV, and HBV and various human oncogenic RNA viruses including HCV and HTLV-1. The mechanism of carcinogenesis underlying these viruses differs from that of the chemical carcinogenesis, radiation carcinogenesis, and enzymatic radical carcinogenesis previously mentioned. Generally the viral protein directly binds to another protein produced by a tumor suppressor gene such as the \textit{p53} gene or \textit{Rb} gene, etc. Otherwise the viral protein acts as a transcription factor and plays a role in the expression of various genes including the oncogene and growth factor, etc., leading to malignant transformation of the cell.

**Genetic Abnormality in Precancerous Lesions**

The genetic abnormalities observed in cancer are frequently detected in the precancerous lesion. Molecular analysis of the multistage carcinogenesis of lung cancer shows that in the smoker, LOH of the 3p and 9p21 genes frequently appears in the bronchial epithelium, which is observed as morphologically normal, after which LOH of the 13q (\textit{Rb}) gene and 17p (\textit{p53}) gene appears. In addition, many chromosomal deletions are detected in 30–40% of ductal hyperplasias in the mammary gland. LOH of the 3p and 9p genes and inactivation of the \textit{p16} gene relates to the carcinogenesis of atypical epithelia of the head, cervix, or esophagus.
The same genetic abnormality observed in intestinal-type gastric cancer is observed in at least 30% of intestinal metaplasias of the stomach. In other words, there is an observed shortening of the telomere, instability of the DIS 191 gene, and mutation of the APC gene and p53 gene (Fig. 1).6 In various adenomas of the large intestine, mutation of the K-ras gene, APC gene, and p53 gene develop frequently. It is the mutation of p53 that plays an important role in the malignant transformation from these precancerous lesions (Fig. 2).7

By introducing the genetic abnormalities found in precancerous lesions into practical medicine, it is possible to distinguish between benign and malignant lesions, and to identify malignant transformation of the precancerous lesion and high risk. While it is possible to decrease the incidence of cancer by improving lifestyle, another method of preventing cancer is based on gene diagnosis.

**Significance and Present Condition of Gene Diagnosis**

Gene diagnosis is of clinical significance in the following five ways. 1) Differential diagnosis between benign and malignant lesions can be carried out. 2) A property diagnosis or quality diagnosis of cancer (malignancy, prognosis, evaluation of susceptibility to chemotherapy and radiation therapy) can be carried out. 3) Diagnosis of the existence and type of cancer can be carried out. 4) Identification of the hereditary tumor and risk diagnosis in the precrisis stage can be carried out. 5) A new treatment for cancer can be developed.

Gene diagnosis in Japan is mainly carried out by identifying 4 items in the hereditary tumor and assessing risk at the precrisis stage. We have routinely practiced molecular pathological diagnosis (gene diagnosis) of digestive tract cancer in cooperation with the Hiroshima Medical Association since 1993.8 According to genetic abnormalities of the p53 gene and
APC gene, 10% of the gastric adenomas from 1,132 cases were diagnosed as gastric adenoma with a high potential of malignancy. Genetic abnormality of the p53 gene and gene instability were detected in 22% of gastric borderline lesions that could not be distinguished histologically as regeneration, metaplasia, or cancer and were diagnosed as cancer. Over expression of the C-erbB2 gene, c-met gene, and cyclin E gene and deletion of the p27 gene were observed in 12% of the 2,822 cases of gastric cancer (80% of these cases were early cancer (T1N0M0)), and were detected as highly malignant gastric cancer. Analysis of genetic instability in 700 cases of gastric cancer showed that 4% of the cases revealed a high frequency of genetic instability (MSI-H) and half of these were identified as simultaneously or separately generated multiple cancers. However, since this is a novel system of gene diagnosis that provides information to enable future diagnosis at the genetic level of gastric tract cancer, there must be further evaluation and follow-up of the prognosis.

Proposal of TNM-G Classification

Based on our current knowledge of genetic alteration and gene diagnosis of cancer, the establishment of a TNM-G method of classification, which includes TNM classification9) and genetic alteration in cancer, is supported by the UICC (Union Internationale Contre le Cancer) committee for practical cancer medicine in the 21st century. Our goal is to construct an international method of evaluation and standard of prognostic factors based on concrete reasoning by integrating information from the TNM system with the analysis of genetic information of cancer. TNM-G classification will support “order-made-therapy” or “tailored therapy” based on a diagnosis of characteristics at the insistence of a clinician and a surgeon. Thus, it is necessary to organize a UICC committee for the discussion of “G” as soon as possible.

Future Prospects

The entire base sequence of the human genome has almost completely been elucidated. Therefore, in the post genome era of the 21st century, both genetic alteration and epigenetic abnormality, especially abnormalities in gene expression caused from the methylation of DNA or acetylation of histone, can be analyzed easily and in a short period of time with new technology including a DNA chip, RNA chip, and protein chip.10) For this purpose, TNM-G classification must be established using the criteria of both genetic abnormalities by cancer and precancerous lesions and epigenetic abnormalities. According to the new “TNM-G classification”, it is possible to formulate a more precise diagnosis, have a precognition of malignancy, metastasis, and recurrence, determine susceptibility to drugs or cancer, and develop new therapies. Once the cancerous patient is introduced to the most suitable method of treatment and prevention based on the characteristics of the cancer, the mortality rate and the morbidity rate around the year 2030 will decrease in this country.

In the case of gene diagnosis, the protection of private information on an ethical level and cancer notification must be reinforced. It is expected that common guidelines on genetic analysis will be formed as soon as possible.

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