Introduction of Liquid Biopsy

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Chapter 01
Super early cancer risk screening tests

PROTEO® Background of Test Development

Early detection with a more accurate method and early treatment are the most effective for “cancer” that is one of the top terminal illnesses in the world. Especially, it is very difficult to predict what kind of course the disease condition of a metastatic cancer will take.

In the current cancer examination, even the latest PET scan has difficulty in detecting lesions of several millimeters. Since it only provides visual information, a biopsy must be performed, which puts a heavy burden on patients with prolonged time and cost. Further, existing blood tests (tumor marker tests), which have been widely spread as simple tests, have low organ specificity and have not been able to identify cancer primary sites yet.

Although diagnostic endoscopy for the digestive system is suitable for gastric epithelial tumors, it is also visual exam and difficult to detect a non-epithelial tumor (tumor on the surface) early.

A progression of cancer is traditionally determined by visually evaluating a size of tumor with imaging studies and supplementarily measuring serum tumor markers (the concentration of a specific protein present in the serum). However, it is unable to find the biological information of the tumor, or whether the tumor cells are actively proliferating or dormant.

In addition, the examination is generally performed at intervals of several months since it is difficult to grasp changes in the size of the tumor in a short period of time. Tumor markers are commonly known to rise due to inflammation and such irrespective of the tumor size or the cancer pathology, and it is said to have little correlation with treatments and changes in the pathology of cancer.

If a test method that can quantitatively and quickly detect cancer-related or Alzheimer-related substances from blood components is developed, it can contribute not only to treatment of cancer and Alzheimer but also greatly to reduction of medical expense worldwide.

Challenges of Liquid Biopsy

Liquid biopsy is ideal as it is minimally invasive; however, it has not been implemented for practical use despite the fact that it has been known as “cancer markers” in the research publications of exosomes, circulating tumor cells (CTCs), and circulating tumor DNA (ctDNA). These marker substances begin to be inactivated as soon as they are taken out of blood. It is also unsuitable for quantitative detection since it is terribly difficult to directly extract the target substance only because the available amount in the blood is extremely small. Moreover, it has been kept away from practical use since the protocol up to the detection is complex and requires a long period of time.
PROTEO® is the “very early cancer screening test” that has been featured in the various media including Japan Broadcasting Corporation (NHK) and received sensational media coverage as groundbreaking, cutting-edge technology capable of detecting cancer from stage 0, and it has been gathering much attention from all over the world.

Up until now, the minimum size of detectable cancer has been from 5 mm to 1 cm. There are as many as 1 billion cancer cells in a 1-cm tumor, and it can still be “too late” even if it is found at this stage. Therefore, studies have been done throughout the world in search of a method that can detect cancer much earlier.

PROTEO® is the technology which can theoretically detect the very early cancer of approx. 1 mm, and it has obtained an international patent for measuring a “nucleosome.” “PROTEO®” has been successful in detecting “molecular-level cancer” for the first time in the world. This is the only test method that can quantitatively measure and quantify the amount of cancer-related substances.

This is the only test method that can conduct cancer examination for a wide range of age from the infant (childhood cancer) to the elderly. Samples can be sent to the medical laboratory via international mail; therefore, the area the international mail covers is the target area for this test. This means that its potential demand can be the whole world’s population of 7.6 billion people.

This screening test can detect the following solid malignant tumors: pancreatic, lung, breast, gastric, umbilical, liver, colon, thyroid, kidney, prostate, uterine, and ovarian cancers. A very small amount of cancer-related substances, which get dissolved in the blood when a cancer occurs, are measured and quantified using a new biochip, PROTEO®. It then determines and classifies the cancer risk into three stages, A (low risk), B (followup required), and C (high risk).
The blood required for this test is as little as 10μL, and it is not affected by meals and such. Thus, this test method is safe with very little physical strain.

Furthermore, the PROTEO® very early cancer screening test shows the differences in measured values so clearly that the results are easily interpreted, which gives a characteristic of very little possibility of misinterpretation.

It can be useful not only as a tool for early detection of cancer but also as a useful test for risks of progression, recurrence/metastasis, treatment effect, etc.

**Diagnosis of Stage 0 Cancer**

A human body is said to have “60 trillion” cells, and “300 billion (0.5%)” of them die daily. “Apoptosis” is a process of “programmed death” of cells that occurs when cells are damaged, not required anymore, or become abnormal.

Cancer cells indefinitely proliferate doubling in number. The size of the tumor that can be found in imaging studies is about 1 cm, but this is why detectable tumors grow quickly. An early stage of cell abnormalities is known to be protected by the mechanism of apoptosis.

Cancer cells are believed to be fragmented to chromatin and nucleosomes, and then to be released in the blood. Since cancer-related substances in the blood contain various information, analysis of those information becomes useful against tumor progression, diagnosing cancer recurrence, determining treatment effects, etc. Thus, its usefulness is attracting attention as a noninvasive test.
Benefits of PROTEO®

1. Very early detection of cancer from stage 0 (treatment is effective).
2. Minimally invasive (very little physical distress).
3. No medical radiation exposure (safe for those who are pregnant or with possibility of pregnancy).
4. No fasting or diet restriction required before the test. (can be tested any time)
5. Blood sample taken at home with self-testing kit (can eliminate disparities in quality of health care).

〈Stable Markers〉

PROTEO® uses nucleosomes (a section of DNA that is wrapped around a core of proteins) as a marker. Nucleosomes are released in the blood by apoptosis (autoimmune); however, the state of nucleosomes, in which proteins and DNA are bound together, is very stable. Therefore, transportation of nucleosomes can be done easily from anywhere in the world.

〈Highly Sensitive Detection〉

Biochips used in PROTEO® measure cancer-related substances by directly absorbing cancer-related substances only. It is possible to detect the target substances at low concentration because there is very little influence of other substances.

〈Label-Free〉

Green fluorescent protein (GFP) is a protein with fluorescence of jellyfish Aequorea victoria and was discovered along with aequorin by Osamu Shimomura in the 1960s. Dr. Shimomura was awarded the Nobel Prize in Chemistry in 2008 for this discovery. Although the discovery of green fluorescent protein (GFP) has greatly contributed to the development of biotechnology, PROTEO® is the world’s first technology of label-free detection which does not require GFP (green fluorescent protein) or any other pigment.

It was featured in “Nanotechnology, Biology, and Medicine” in October 2013.
It was featured in the online scientific magazine “Scientific Reports” published by the Nature Publishing Group on May 21, 2015.

PROTEO® is performed by directly absorbing cancer-related substances only. Even low concentration of target substance can be detected since there is no influence by other substances. Highly precise detection and direct quantitative detection are performed by omitting complicated pre-processes.
Place one drop of blood component (serum) from the centrifuged blood on the metal chip.

Cancer-related substances, nucleosomes, react with and get absorbed by silver peroxide meso...

Autofluorescence of absorbed nucleosomes are observed by applying light.
Fluorescence image of the cancer-related substances
Because crystals of cancer-related substances released in the blood are derived from cells related to apoptosis, the contained amount in a healthy body or a benign tumor is small while a large amount is contained in the blood of the diseased. Therefore, there is a characteristic that fluorescence appears much more in malignant tumors.

**A Simple Procedure**
PROTEO® requires no complex procedures, and all protocols can be completed within 10 minutes.

Since the PROTEO® biochip is a new highly sensitive assay method, cancer-related substances (nucleosomes) can be quantified and detected quantitatively.

Unlike traditional DNA, microarray and microfluidic chips, cancer-related substances (nucleosomes) in the blood were directly detected label-free successfully without using probes such as DNA, antibodies, fluorescent substances, etc.

The detection of cancer-related substances at an extremely early stage, which exceeds the existing test methods, is expected to bring breakthrough results in the field of cancer diagnosis such as physical examination, treatment effect, and diagnosis of cancer recurrence.
We succeeded in the world’s first synthesis of “quantum crystals” and “silver peroxide meso crystals” (three-dimensional self-assembled crystals), which has the effect of specifically absorbing proteins on the surface of biochip. This is a novel substance with a new concept.

Novel substances that absorb nucleosomes

Quantum Crystals
(three-dimensional self-assembled crystals)
Results of PROTEO®’s Clinical Trials

Silver Peroxide Meso Crystals
International regulations
There are international regulations on every means of transportation of infectious substances (air, rail, road and marine transport as well as mail), and they are released as a form of model regulations based on the recommendation of the United Nations Committee of Experts on the Transport of Dangerous Goods (UNCETDG) which is the committee of the United Nations Economic and Social Council. The UN model rules are reflected in the international laws through the international model agreements.

For these reasons, global medical testing services have not been actualized so far.

We have established the dried serum technology by separating serum from the blood as a way to solve these problems. This enables safe transport of dried serum.

By enabling transport of dried serum, it is possible to perform a stable test whose accuracy is not adversely affected by the condition of transportation.

Permission by Individual Countries
There is nothing in the category of medical equipment in Japan that is equivalent to a biochip used for PROTEO®, and it has been implemented as a doctor-led clinical trial (Effectiveness Trial). As the current application status of overseas biochip medical devices, the classification of DNA biochips is approved for category 1 and 2. We aim for obtaining approvals corresponding to qualifications for medical device application in each country.
The technology of PROTEO® has been successful in basic research and clinical trials, and the results have been published, including the following.

May 2015  “Scientific Reports” published by the Nature Publishing Group
October 2013 “Nanotechnology, Biology, and Medicine"
July 2013  “Royal Society of Chemistry”