

Basic Application Research on a Method for Earliest Diagnosing Cancers

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Abstract

Background and Objective: The author of article created a molecular method for diagnosing cancers in earliest stage of carcinogenesis to treat the cancer well as the cancer early diagnosis has been poorly developed.

Method: Referenced the scientific achievements of cancer research up to now, the author designed the novel creation. Then the value of method for earliest diagnosing cancers has been accepted by the international medical societies.

Results: Using two different normal Rb protein antibodies and two different normal p53 antibodies, develops antigens and antibodies immune reactions respectively, with specimen of Rb proteins and p53 by special analysis method. After comparing the two reactions of Rb proteins' and the two reactions of p53's by application of Affinity Chromatography and Flow Scintillation Counter, the structures and functions of Rb genes and proteins, p53 genes and proteins can be judged. So the cancers can be diagnosed earliest. This invention for diagnosing cancers in earliest stage of carcinogenesis could be used for many kinds of cancers to be diagnosed in the same method.

Conclusions: The new research method of molecular biology for earliest diagnosing cancers used by author in this paper is worth to be developed further and applied in clinical practice. It is first reported by the author in this article. Which is identified through a MEDLINE search of the English-language literature on the title or on the key words. The novel method has been proved valuable when it was accepted by international oncology societies.

Keywords: diagnosis; p53; retinoblastoma protein; immunoassay; structure of protein.

1.0 Introduction

The cancers have caused heavily harms to the mankind. Among them, the cancers caused the patients disablement and worst ache, even caused the patients early death. But, up to now, the mortality and morbidity of majority cancers have not been controlled. The mortality and morbidity of some kinds of cancers even are going up. As the cancer control has not reached satisfied achievements. The early diagnosing cancer has been being much too important and imperative. If one earliest diagnosing method could diagnose the cancers in situ or before the stage of cancers in situ.

The cancers treatment might almost be cured. The author of this paper has been studying and learning the oncology from his graduate study time and his working time when he created a new method for diagnosing cancers in situ or before the stage of cancers in situ. This paper is a principle creation for broad purpose to diagnose early stage of

carcinogenesis of precancerous lesions or cancers in situ. The principle creation has not been elaborated and is not elaborated just for broad purpose. That is because the molecular method of the creation is for many kinds of cancers to be diagnosed. The specificity is not limited to one cancer, but for lots of cancers' earliest diagnosis. The sensitivity should be highest.

There are no reports about the creation or related research and clinical application. Which is identified through a MEDLINE search of the English-language literature on "A Novel Proposal for Earliest Diagnosing Cancers" or the key words of this paper. Though the author has not the conditions and the chances to apply the novel creation into practice. But the author thinks that the creation must be very useful!

2.0 Method

The article is a research article. The research method is man-made meta-analysis, referenced the scientific achievements of cancer research up to now. Then the author designed the novel strategic creation for earliest diagnosing cancers based on the following mechanism. Then the value of method for earliest diagnosing cancers has been accepted by the international medical societies after the abstract was exchanged at the international academic conferences.

The cancer research displays, that the tumor suppressor genes play a very important role in the normal cell biology, physiology and the cancers development. Up to now, there are lots of tumor suppressor genes have been found. Their abnormal functions can cause cancers development. The retinoblastoma gene (Rb gene) and the p53 gene are the earliest found and the most important tumor suppressor genes.^{1,2,3} The reasons to say the Rb gene and the p53 gene are the most important tumor suppressor genes are that their abnormalities have been found in lots of cancers and precancerous lesions and their abnormalities can cause lots of cancers and precancerous lesions.^{4,5,6,7,8,9,10,11}

The abnormalities of Rb gene and the p53 gene are expressed in their abnormal proteins which have function and structure abnormalities. Losing of Rb proteins and p53, reduced amount of Rb proteins and p53, dysfunctions of the Rb proteins and p53, abnormalities of configuration of the Rb proteins and p53, et al. are the patterns of abnormalities in Rb proteins and p53.

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Rb genes, p53 gene and their abnormalities of proteins are the molecular pathologic changes of carcinogenesis. If one part of tissues of human body had physiologic, pathologic or (and) clinical abnormalities of precancerous lesions before or in the stage of cancers in situ. And at the same time, if the abnormalities of Rb proteins and p53 were found in those tissues. The cancer in situ or the earliest stage of carcinogenesis in this part of tissues could be diagnosed. Therefore, if the abnormalities of Rb proteins and p53 were tested in one part of human tissues, where has clinical abnormalities but before the stage of cancer in situ. The earliest carcinogenesis tissues from the stage of molecular level could be diagnosed. There is one period of time to go when tissue cells change from abnormalities of Rb proteins and p53 to cancer. But the tissue cells must change to cancer if the life model of patient is not going to be changed into better. So diagnosing the abnormalities of Rb proteins and p53 is the earliest method to diagnose cancers. So the treatments, including prevention, operation, are the earliest and much too effective and curable.

Because it is easy to test the abnormalities of molecular configuration or stereochemistry construction of Rb protein and p53 in all cancers by this proposal, comparing to test the abnormalities of Rb gene and p53 gene one type by one type in conventional method. So testing the abnormalities of molecular configuration or stereochemistry construction of Rb protein and p53 is easy to do in experiment and the clinical practice.

Girod SC, *et al.* had considered to use the alterations of p53 and Rb gene as a potentially useful prognostic 'biomarkers' in oral carcinogenesis.¹² While Kawakubo H, *et al.* had assumed the genetic alternate expression of Ki67, p53, cyclin D1 and pRB may be useful biomarkers for assessing the risk of developing esophageal cancer.¹³ Though they considered to use p53 and pRB as a biomarkers. But they only were assessing a single cancer and they used the genetic alternate expression of p53 and pRB as biomarker. Which are much too complicated and difficulty or expensive to be used clinically. While my invention is intended for many kinds of cancers to be diagnosed in a single way and only assessing the functional abnormalities of Rb protein and p53 in a special easy way.

3.0 Results: The novel molecular creation for earliest diagnosing cancers

3.1. Principles of this creation

The author created there are two kinds of antibodies to be used. One kind of antibody for this diagnostic method (The author called: -antibody) is produced respectively by immunizing the peptides expressed by one exon of Rb gene or one exon of p53 gene. Which the peptides and the exon of Rb gene or the exon of p53 gene are most difficult to be abnormal or no abnormality at anytime. While the second kind of antibody (The author called: -antibody) is produced respectively by immunizing the peptides expressed by one exon of Rb gene or one exon of p53 gene. Which the peptides and the exon of Rb gene or the exon of p53 gene are the most easily to be abnormal.

Rb's -antibody and p53's -antibody respectively react, at the same time, with the Rb proteins in the same amount of

suspected cancer specimen. At the same time, p53's -antibody and p53's -antibody respectively reacts, at the same time, with the p53 in the same amount of suspected cancer specimen. Which all are immune reactions of antigens and antibodies and which the Rb proteins and p53 in the suspected cancer specimen are labeled respectively by radioisotope radio-sulfurs or radioisotope radio-iodium. Then tests the intensity of radioactivity of Rb proteins and p53 using the proper technology respectively. So that we can analyse if the structures of the Rb proteins and p53 are abnormal. So that we can analyse if the Rb genes and p53 genes are abnormal. If the Rb genes, p53 genes and the Rb proteins, p53 are abnormal. The organ where the specimen were got is or will be cancer. It shall be cure in proper ways. By the immune reactions of antigens and antibodies, how we knows if the Rb genes, p53 genes and the Rb proteins, p53 are abnormal?

Just for the concise, for example, we use the Rb gene, its proteins and related experiments to explain how to infer if the Rb genes, p53 genes and the Rb proteins, p53 are abnormal. Because at this point, the related experiments for Rb genes, p53 genes and the Rb proteins, p53 are similar. After the immune reactions are done by -antibody and -antibody with Rb proteins respectively. We can test the intensity of radio-activity of Rb proteins. So can we analyse the quality of the Rb proteins.

Followings are ways to analyse:

- (1), If the intensity of -antibody by the radio-activity immune reaction is lower than the intensity of radio-activity by the immune reaction of -antibody or there is no intensity of radio-activity by the immune reaction of -antibody. We can say that this patient of specimen has the abnormalities of functions and structures of Rb proteins. So the related Rb genes are also abnormal.
- (2), If the intensity of the radio-activity by the immune reaction of -antibody is lower than the intensity of the radio-activity by the immune reaction of -antibody or there is no intensity of radio-activity by the immune reaction of -antibody. We can say that this patient of specimen has the abnormalities of functions and structures of Rb proteins. So the related Rb genes are also abnormal.
- (3), If there is no intensity of radio-activity by the immune reactions of -antibody or -antibody. We can deduce like the above mechanism that we can not test the immune reaction, and there is no Rb protein in the patient's specimen. We can say that this patient of specimen has the abnormalities of functions and structures of Rb proteins. So the related Rb genes are also abnormal.
- (4), If the intensity of radio-activity by immune reaction of -antibody is the same as the intensity of radio-activity by immune reaction of -antibody. We can say the Rb genes and Rb proteins are normal. This patient of the specimen may not be the early carcinogenesis or cancer in situ. We should test again the above experiment after a proper interval time.
- (5), If the intensity of radio-activity by immune reaction of -antibody was the same as the intensity of radio-activity by immune reaction of -antibody, at the same time the absolute weight value of Rb proteins are lower than normal or there

are other exons of Rb gene are abnormal. We can say theoretically that this patient of specimen had the abnormalities of functions and structures of Rb proteins. So the related Rb genes were also abnormal. But on this occasion, the chance may be almost none. And which this proposal does not assay. This patient of the specimen shall be tested again the above assay after a proper interval time or the other assay method should be used. But the author may say there are no other assay methods more precise than the author invented method of assay.

Using the same method, we can test, analyse, and infer if the p53 genes and the p53 are abnormal.

3.2. Principles of direction for treatment

After we test and know the Rb genes, p53 genes and the Rb proteins, p53 are abnormal or normal. We can analyse and direct how to treat the patients according to the test results. The methods to infer and direct treatments are as follow:

(1), If the Rb genes, p53 genes and the Rb proteins, p53 on the precancerous lesion are all abnormal. The early operation on the precancerous lesion is curable at this time.

(2), If the Rb genes and the Rb proteins or the p53 genes and the p53 on the precancerous lesion is abnormal. The clinical intensive observation and intensive prevention are needed for the patients. And at the same time, the regular physical check-ups and regular test with this diagnosing method must be applied. The early operation on the precancerous lesion may be curable at this time, depending on proper clinical management.

(3), If the Rb genes, p53 genes and the Rb proteins, p53 on the precancerous lesion are all normal. The clinical observation and prevention are needed for the patients.

According to the mechanism of this new proposal for earliest diagnosing cancers and its test, inference method, we can diagnose the molecular abnormalities of proteins or genes of carcinogenesis of patients in early stage, but no tumor change on the precancerous lesions. That is the much too earliest stage of carcinogenesis. So the diagnosis is much too earliest. Therefore, reaches the curable objective with the earliest operation on the patients after the diagnosis.

3.3. Test processes of the assays

According to the above mentioned mechanism and by the modern experimental conditions and technology, the author invented the new experimental principles of process for the earliest diagnosing cancers. It is worth to be said that all the experimental processes are easy to do at modern time. Also just for the concise, the author uses the Rb proteins and related experiments for example to express what the new experimental principles of process for the earliest diagnosing cancers are. Because at this point, the new experimental principles of process for Rb proteins and that for p53 are similar.

3.3.1. Antigen preparation

In order to produce the antibodies of Rb proteins for these immune reactions, the proper peptides transcribed by exons

of Rb gene must be prepared. Now, the amino-acids sequence of Rb protein is clear, The researchers shall only choose the proper peptides by the above mentioned principles. The peptides can be synthesized at present and their antibodies have become commercials.

3.3.2. Preparation of antibodies

Up to now, there are lots of methods to produce the antibodies for Rb proteins. And there are many kinds of monoclonal antibodies and multi-clonal antibodies. There are different methods to produce monoclonal antibodies and multi-clonal antibodies. According to the peptide of Rb protein, we can produce related antibodies. All the monoclonal antibodies and multi-clonal antibodies can be used in these experiments. But it is much too cheap to produce multi-clonal antibodies.

The principle of processes to produce antibodies is as following: Preparation of peptides → adding some materials to the peptides for working as full function antigens → antigens immune the hares of medical usages → extraction of the antibodies → prove the antibodies to be right ones and purify them.

3.3.3. Preparation of specimen

The specimen suspected as cancer tissues are mainly the cancers in situ and precancerous lesions in patients. We shall biopsy them as early as possible. Or, we can get them by other ways, for example, operations, endoscopies examinations, *et al.* And masses scans for physical examinations of normal persons are the good way to get specimen. Also the modern methods or technologies to get the specimen should be used.

3.3.4. Labeling of the specimen by radioisotopes

There are two kinds of methods to label the specimen by radioisotopes. One is labeled by radio-sulfurs. The other is labeled by radio-iodium. When labeling radio-sulfurs, we only need to culture the cells of live specimen with radioactive sulfurs in a short time. Which can label the radioactive sulfurs on the Rb proteins. When labeling the radio-active iodium, we must grind the cells of live specimen. Then, the radio-active iodium are labeled directly to the Rb proteins. Which method is better to label the radioisotopes? The author thinks it is better to use radio-active sulfurs.

3.3.5. Application of Affinity Chromatography

The radioisotopes labeled antigens of Rb proteins in the same amount of suspected cancer specimen react respectively with -antibodies and -antibodies on the Affinity Chromatography. Which reaches the aims of purifying and extracting the radioisotopes labeled Rb proteins. So that the further research and usage can be finished correctly.

3.3.6. Application of Flow Scintillation Counter

According to the directions of this equipment, measure the intensity of the radioactivity of Rb proteins which were extracted and purified by the Affinity Chromatography using the immune reactions of -antibodies and -antibodies. When measuring, we shall use blank control group.

3.4. Clinical usage probe

The author has worked out the whole assay principles of process of this new invention for the earliest diagnosing the cancers by referencing the present oncology research achievements. The author can say theoretically that this assay of principles of process can diagnose clinically the cancers on the lesions of patients and (or) normal persons with great value. But, in order to prevent and cure the cancers more scientifically and more precisely. It is necessary to research on random clinical case-control trials(RCT) for the test of pre-clinical practice. So we can prove this invention is scientifically effective when it is used in clinical practice. It is better to use the same kind of mass patients of cancer for the earliest diagnosing and treatment in random clinical case-control trials. The control group with precancerous lesions are tested their abnormalities of Rb proteins and p53.

They are not treated like the research group. But live like before, treated with conventional therapies. The research group, when are found their abnormalities of Rb proteins and (or) p53 in precancerous lesions, treated with above-mentioned anti-cancers therapies at once. Then comparing their mortalities and morbidities of cancer and test their significances statistically. So we can prove if this earliest diagnosing method is right and scientific.

4.0 discussion and Conclusions

The achievements of modern oncology consider the suppressing abnormalities of Rb genes, Rb proteins and p53 genes, p53 are the primary reasons of carcinogenesis. Therefore, the author of this paper, created that testing the abnormalities of Rb proteins and p53 in precancerous lesions of the patients by radioisotopes labeling and methods of immunoassay. So that we can prove the patients have been being in the earliest stage of carcinogenesis. Therefore, the cure, better treatment and prevention could be reached for the patients. So the significances are great. This method has great value theoretically. But before it is used in clinical practices. Researching precisely and doing lots of works to build the standards of all stages of assay are needed. At the same time, we should use the computer to digital and analyse the data of assay. Which finally make the modern scientific equipment to earliest diagnose the cancers for clinical usage and commerciality.

Though the paper is only a process of invention. But it shall be easy to understand by the achievements of modern cancer research. Theoretically to comment, the invention is much too scientific. Because the carcinogenesis is multi-steps. That the conclusion based on only one positive of abnormality of protein(Rb proteins or p53) to say the cancer is diagnosed is less scientific than the conclusion based on two positives of abnormalities of the proteins (Rb proteins and p53). But it is not necessary when the conclusion based on positive of more than two abnormalities of the tumor suppressor gene proteins (Rb proteins and p53 and others). The reasons why to assay the Rb proteins and p53 as the proteins of tumor suppressor genes are that the retinoblastoma gene (Rb gene), Rb proteins and the p53 gene, p53 are the earliest found and the most important tumor suppressor genes and proteins. Further more, the Rb gene and p53 gene and their

abnormalities of proteins were found in most of the cancers and precancerous lesions.

This molecular method can be used for the earliest diagnosing cancers, like the esophagus cancer, gastric carcinoma, colorectal cancer, small cell lung cancer, uterine cervix cancers, carcinoma of the breast, osteosarcoma, oral squamous cell carcinoma, *et al.* All which the Rb genes and p53 genes and their abnormalities of proteins were found in these cancers or their precancerous lesions. It is clear that their precancerous lesions or cancer lesions are specific sites (tissues) which should be biopsied rightly and tested. And masses scans for physical examinations of normal people using this assay method are the good way to get specimen to diagnose the earliest precancerous lesions of normal peoples.

According to this test process, the other tumor suppressor genes and their proteins, when necessary, can also be measured.

Further more, in this creation, the author created theoretically a new research molecular method which should be paid more attention for further research and application. It uses different immune reactions to probe if the function and structure of proteins of human cells are normal or abnormal at the same time. So as to infer if the function and structure of human cells related genes are normal or abnormal. It is a new, concise, and special research method of molecular biology.

It has been proved the proposal is valuable that the summery of the paper was accepted and published in The Proceedings of UICC World Cancer Congress, 2006.¹⁴ And the abstracts of this paper have been accepted and published on two important Journals, the PSYCHO-ONCOLOGY and ANNALS OF ONCOLOGY.^{15, 16}

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