NPO　Meeting which considers integrated medicine and health
21, Uenosono-cho, Kagoshima-shi-4
TEL:0120-661-566 / FAX:0120-661-589
E-mail: info@tougouiryou.jp
URL:http://www.tougouiryou.jp

Noted in the field of integrative medicine

Enzymatically digested

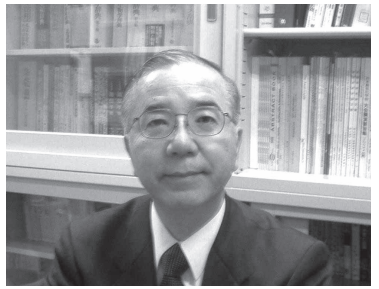
low molecular weight fucoidan

derived from seaweeds

- Focused on anticancer effects -

Sanetaka Shirahata
Professor
Laboratory of Cellular Regulation Technology
Department of Bioscience and Biotechnology
Faculty of Agriculture
Kyushu University

Introduction



Prof. Sanetaka Shirahata, Laboratory of Cellular Regulation Technology, Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University

A medical practitioner's words prompted me to start my research on anticancer effects of low molecular weight fucoidan.

It was the autumn of 2002. The medical doctor working in Fukuoka Prefecture in Japan came to my laboratory, and said in an excited tone "The cancer of my patient disappeared!"

Although cancer is intractable illness, it is possible to cure it by proper treatments. When I heard the words of the doctor, I thought that the cancer had been found in the early stage and some medical treatment had been probably effective.

However, the patient was over 70 years old and it was the case that progressive cancer of right lung caused metastasis to left lung. The size of the caner reached to 4 cm and he was declared from his hospital that "There was no further treatment to be selected in Western medicine. Your life expectancy is about 3 months". For the patient, the doctor tried treatments in the field of the "integrative medicine" which combined alternative medicine, such as dietary therapy, Eastern medicine and a supplement treatment, and he also recommended the patient to drink a supplement of low molecular weight fucoidan.

Although there were few clinical cases and the supplement was new for the doctor, the effect of the supplement was drastic far beyond his anticipation. The patient was bedridden mostly due to a severe cough. However, the cough was subsided three days after starting to drink, and the patient could take a walk after half a month. When the patient received the examination of tumor markers and MRI after 3 months, the values of tumor markers returned to the normal level and the cancer completely disappeared.

Low molecular weight fucoidan usually means a liquid supplement, which consists of extracted and enzymatically digested slime fucose-rich polysaccharide derived from brown algae, such as mozuku and wakame seaweeds. Fucoidan was discovered in 1913 by H. Z. Kylin, a Swedish scientist and many papers have been published on its structure and physiological activities. At the 55th General Meeting of Japanese Cancer Association held in 1996, a presentation on research of the apoptosis (natural cell death) induction effect of fucoidan was first reported, and some researchers noted the research in Japan.

Based on such a background, when I heard the talk on clinical effect of the low molecular weight fucoidan, I immediately sensed that the research on anticancer effects of fucoidan would be important. On the other hand, I felt that more evidences would be needed to promote the research.

Therefore, I decided to cooperate with many medical doctors in the field of integrative medicine to accumulate clinical instances as well as promoting basic researches on low molecular weight fucoidan in 2003.

The results of basic and clinical researches on low molecular weight fucoidan, which have been continued for more than 10 years, were briefly summarized in this book as intelligibly as possible. The content includes structures and 3 representative functions of fucoidan such as cancer-specific apoptotic cell death induction, suppression of tumor angiogenesis, and enhancement of tumor immune system, and scientific fruits presented in the meetings of Japanese Cancer Association and World Cancer Congress.

It is my great pleasure if this book is helpful for patients suffering cancers and their families to have a hope.

C O N T E N T S

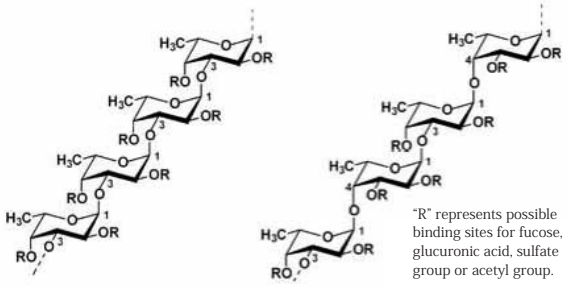
Chapter 1 Basic Knowledge of Low Molecular Weight Fucoidan

■ Fucoidan is an ingredient of *numeri* of seaweed.

When you were child playing in a rocky beach, don't you have an experience of slipped on seaweed? Seaweeds (brown algae), such as mozuku seaweed, wakame seaweed and kelp, are covered on its surface with slimy (*numeri*) materials.

Sulfated polysaccharides contained in the *numeri* materials are "fucoidan" More scientifically; sulfate groups connected to fucose, a type of sugar, is linked to each other forming long chains as polysaccharides and are generically called fucoidan.

A fundamental structure is as follows.



• Basic structure of fucoidan

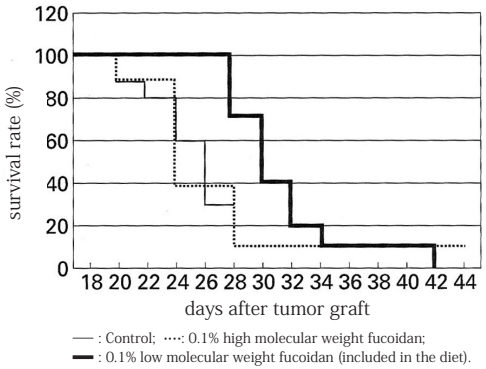
The structure of fucoidan depends on the kinds of seaweed to extract. In the research, we use fucoidan extracted from the mozuku seaweed from the Kingdom of Tonga. The mozuku seaweed was selected because it contains about 5-8 times more fucoidan compared with wakame seaweed or kelp.

Fucoidan is extracted with organic acid from the mozuku seaweed which grew in clean Tonga seawater and desalted prior to use. Then, the crude fucoidan with molecular weights of 200,000-800,000 are digested to less than 500 using a specific enzyme.

■ Anticancer effects of low molecular weight fucoidan

The molecular weight which a human body can fully absorb is thought to be less than 3,000. Therefore, the low molecular weight fucoidan is expected to be absorbed with high efficiency from the digestive system to the body.

Another reason to reduce the fucoidan size is related to suppressive effects on tumor growth. The following graph shows the effects of high- and low-molecular weight fucoidan diets on the survival rate of the colorectal cancer cell inoculated mice.



• Survival ratio of mice grafted with colorectal cancer cell

Although it turned out that high molecular weight fucoidan prevents a tumor enlargement in an early stage, the strong tumor suppressive effect as a whole could not be recognized. While low molecular weight fucoidan could prolong its tumor suppressive effect and the life extension effect in terms of post inoculation days was clearly recognized.

■ The safety assessment of low molecular weight fucoidan to human body

Prior to low molecular weight fucoidan research, we assessed the safety of low molecular weight fucoidan to human body. This is a method called the "Ames test" developed by an American Professor Ames in 1975, and is a test to evaluate

whether contained ingredients cause mutation in the DNA of living organisms.

A low molecular weight fucoidan extract was tested by this method, and we found that neither gene mutation nor mutation inducibility were observed.

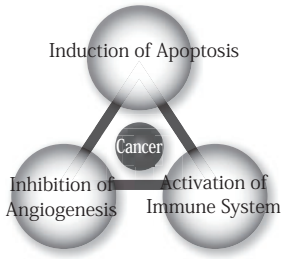
Moreover, we tested whether there would be any danger to be transformed to a genotoxic agent during metabolizing process in the liver and we found that there was no such sign. Furthermore, we did not see any abnormalities in the acute toxicity test using a mouse and thus we judged that low molecular weight fucoidan is safe for a human body.

■ Three major effects of low molecular weight fucoidan

There is a question of how low molecular weight fucoidan acts against cancers. The effects which became clear by research are the following three points.

1 Induction of Apoptosis
2 Inhibition of Angiogenesis
3 Activation of Immune System

Although the following chapter explains in detail, three major effects can be summarized as that low molecular weight fucoidan will directly act on a cancer cell, and will lead a cancer cell to a natural death (apoptosis). Moreover, it suppresses the angiogenesis for nutrients supply to a cancer cell, and enhances a patient's own immune system.



• Three major effects of low molecular weight fucoidan

Although an antineoplastic drug generally gives damage even to a normal cell, low molecular weight fucoidan acts only on a cancer cell. That is, while it is "an antineoplastic agent without side effects", it turns out by our accumulated research data that the function of healthy cells is strengthened more.

■ Known functions of low molecular weight fucoidan until now

Fucoidan has not only an anticancer activity but also a various function. Altogether fucoidan is thought to be a superior food ingredient for our health. The elucidated functions of fucoidan are summarized below.

Elucidated fucoidan functions

[Anti-tumor and anti-carcinoma activity]

1) Enhancing effect of immune system
   - Macrophage activation and interleukin (IL) 12 induction
   - NK cell activation
2) Apoptosis
   - No cytotoxicity on normal lymphocytes
   - Dose dependent induction of apoptosis on cancer cell
3) Anti-angiogenesis
   - Inhibition of VEGF binding to receptor
   - Inhibition of VEGF expression and secretion
4) Metastasis / invasion
   - Inhibition of MMP-2, -9 expression, secretion and activation
5) Prevention of cancer-cell adhesion
   - Preventive effect of sugar chain adhesion (inhibitory activity of sulfatide-secretin binding)
6) Prevantion of cancer growth and effect of life prolongation in mouse.

[Anti-*Helicobacter pylori* • anti-ulcer effect • improvement effect of stomach discomfort]

- A sulfate group captures *Helicobacter pylori*.

- Protection of a mucous membrane
- Anti-inflammation effect

[Anti-allergic effect • anti-inflammatory effect]

1) Regulation of Th1 / Th2 ratio
   - Th2 suppression (IL-2, -3, -5 suppression), suppression of mucus producing cell, decreasing eosinophil
   - IL-4, IgE, anti-BSA specific IgE suppression, suppression of histamine release

2) Suppression of inflammatory cell control
   - Improvement of atopic dermatitis model mouse symptom

[Up-regulatory effect of liver functions] Enhancement of HGF production

- Improvement of GTP, GOT and γ-GTP values

[Anti-lifestyle-related diseases]

- Improvement of blood cholesterol level, neutral fat value, blood sugar level and anti-obesity effect

[Anti-diabetic effect]

- Enhancement of sugar uptake in muscle cells
- Suppressive effect of hyperglycemia in diabetic model mouse

[Anti-viral effect] Herpes, HIV, etc.

- Induction of apoptosis in HTLV-1 infected cells, increasing neutralizing antibody titer

[Anti-bacterial effect]

- Suppression of food poisoning bacterial growth (Salmonella)

[Anti-oxidative effect] Anti-aging

- Elimination of reactive oxygen species

[Suppressive effect of blood coagulation] Anti-arteriosclerosis

- Conversion of viscous blood to a smooth state

[Lustrous skin formation] moisture retention, wrinkles, stain, dullness

- Wrinkles control, suppression of collagen degradation, anti-oxidative effect
- Suppression of hyaluronic acid degradation, enhancement of hyaluronic acid synthesis
- Suppression of hyaluronic acid synthase degradation, suppression of histamine release
- Enhancements of skin moisture retention, elasticity maintenance and wound healing
- Improvements of skin hygroscopicity and water retention

Chapter 2 Fruits of Research on Low Molecular Weight Fucoidan

■ Mechanism of carcinogenesis

This chapter explains 3 major effects of low molecular weight fucoidan. Beforehand, let's introduce briefly about the mechanism in which a malignant cancer cell appears.

Our body contains about 60 trillion cells which are classified into over 200 kinds of cell types making up each organ and finely regulated each other to efficiently function as a body. These cells are renewed in a fixed cycle. For examples, a tissue such as the skin is renewed in about 28 days and a bone is renewed in about four months. Although the phenomenon of older cells die out is considered to be a natural event, in fact, the instructions of "self-destruction" are incorporated in the genetic information, and thus there is a mechanism that any cell will "choose to die toward self destruction fate" when a given cell exceeded a defined period.

Moreover, also when an accident happens to a cell, the switch for self-destruction will be turned on. The phenomenon of natural cell death as above is called "apoptosis" with a medical jargon.

In familiar examples, phenomena will depend on the apoptosis induction as that losing a tail in the growing process of a tadpole to a frog, or the web existed in the embryonic stage is also lost.

Now, in order for a cell to be renewed, it is necessary to replicate exactly the same genome as before, but the miscopy of the genome may occur by various reasons. As a result, the cells which should have aged and died, now gain an infinite life and start to grow. This is the cancer cell.

In fact, about 5,000 cancer cells are being produced even in a healthy adult. However, a self-defense system called the tumor immunity mechanism of a living body will recognize the cancer cells as foreign substances and eliminates them.

However, when immune strength decreases due to aging, the imbalanced poor eating habits, smoking habit, stresses, etc., cancer cells will survive and will gradually gain malignant cancer characteristics.

There are six troublesome powers in a malignant cancer cell.

1. Power to grow selfishly
2. Power to ignore the cell division arrest signal liberated when a different cell contacted each other
3. Power to ignore the apoptotic signal
4. Power to indefinitely live without aging
5. Power of angiogenesis in order to monopolize nutrition
6. Power to spreads in the body through metastasis and invasion

Especially acquisition of the last metastasis and invasion power is the worst malignant characteristics, and when a cancer is converted to such state, it is diagnosed as the stage 3 or 4.

In contemporary medicine, operations, anti-neoplastic drugs, and radiation therapies are considered as the three major medical treatments against malignant cancer.

■ Apoptosis induction leading to cancer cells self-destruction

Now, it will be explained the effect of low molecular weight fucoidan, one of the supplements.

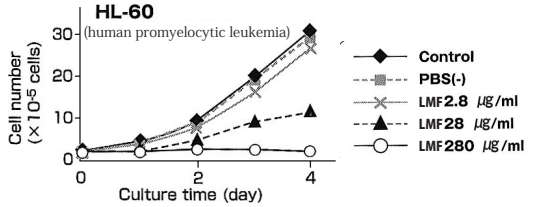
There is "apoptosis induction effect" as a characteristic property of low molecular weight fucoidan. Strikingly, low molecular weight fucoidan acts only on a cancer cell specifically and leads to apoptosis.

In our research, low molecular weight fucoidan of various concentrations was added to healthy cells and cancer cells which resulted in an observation of apoptosis induction.

The amounts of cellular DNA decreased in the apoptotic process. The occurrence of apoptosis was investigated by the flowcytometry. The flowcytometry is the equipment for measuring the amount of DNA by exposing laser beams to the cell.

As a result, it was found that the application of low molecular weight fucoidan to normal cells, apoptosis was not observed at all. Therefore, it can be regarded as that there is no cytotoxicity to normal cells, and it will not damage to all other cells like the side effects exerted by the anti-neoplastic drugs.

When low molecular weight fucoidan of various concentrations was added to the human leukemic HL-60 cells, a kind of cancer cell, it will induce apoptosis and apoptosis inducibility is increased dose-dependently.



• Suppressive effect of enzymatically digested low molecular weight fucoidan on human non-adherent cells growth.

Furthermore, the extract of low molecular weight fucoidan is added to fibrosarcoma cell line HT 1080, a kind of cancer cell, and observed during 48 hours.

Low molecular weight fucoidan treated cancer cell became round gradually, ceased cell movement, and cell contents had begun to leak like a balloon. That is, we confirmed that the cells initiated apoptosis.

Although observed phenomena are correct, identification of which ingredient in fucoidan is responsible for the induction of apoptosis is not finalized yet as it is consisted of a complex polysaccharides despite the researchers in and outside the country including us are working to specify the active substances.

However, if an active ingredient can be specified, development of more effective medicinal drugs and supplements is expected.

■ Suppression of tumor angiogenesis to stop nutritional supply for cancer cells

The cancer cell is very tough and clever. In order to grow, the cells require a large amount of nutrition for own use. To accomplish this, a cancer cell will synthesize blood vessels. This is called "angiogenesis."

A cancer cell extends new blood vessels, and secures the nutrition supply for own growth.

Like a spider, the cancer becomes larger, more new blood vessels are formed and extended and more nutrition is absorbed avariciously.

As a result, a pocket called a malignant tumor is established and sufficient nutrition cannot be supplied to the patient's body. Even if the meals are taking as usual, there are many cases that the patients become thin and emaciated. When physical strength is lost, the resistance to cancer cell growth will also be lost.

In order to prevent cancer growth, it is required not to allow the new blood vessel formation for nutrient supply. If nutrition is no longer supplied, consequently the vigor of cancer will also become weaker and it will reach to the state of a "necrosis."

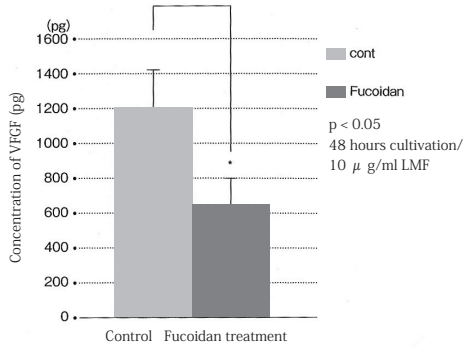
Although the ingredient of the shark cartilage has been known as a supplement with anti-angiogenesis effect, we experimentally showed that fucoidan contains the ingredient having the same effect as the shark cartilage.

This experiment is designed to apply low molecular weight fucoidan to a human cervical cancer cell line HeLa cell (hereafter, cervical cancer cell).

The vascularization will take place when a cancer cell secretes the blood vessel growth stimulator VEGF (vascular endothelial cell growth factor). We confirmed that the VEGF expression was evidently suppressed when low molecular weight fucoidan was applied to cervical cancer cell.

Although the figure shows an investigation of the VEGF levels upon addition of low molecular weight fucoidan with a concentration of 10 μg/ml to cervical cancer cells, it turned out that the level of VEGF is significantly suppressed compared with that of without low molecular weight fucoidan.

Furthermore, it turns out that low molecular weight fucoidan suppresses not only VEGF production but also angiogenesis.



• Enzymatically digested low molecular weight fucoidan suppresses angiogenesis induced by HeLa cells derived from cervical cancer.

These results were summarized in the paper entitled [Enzyme-digested Fucoidan Extracts Derived from Seaweed Mozuku of *Cladosiphon novae-caledoniae* Kylin Inhibit Invasion and Angiogenesis of Tumor Cells], and published in an international scientific journal "*Cytotechnology*."

■ Stimulatory mechanism of tumor immune system

It was mentioned above that there are about 60 trillion cells in a body. Preventing the mutation of a normal cell to a cancer cell depends on the "anti-oncogenes" associated with the apoptosis system.

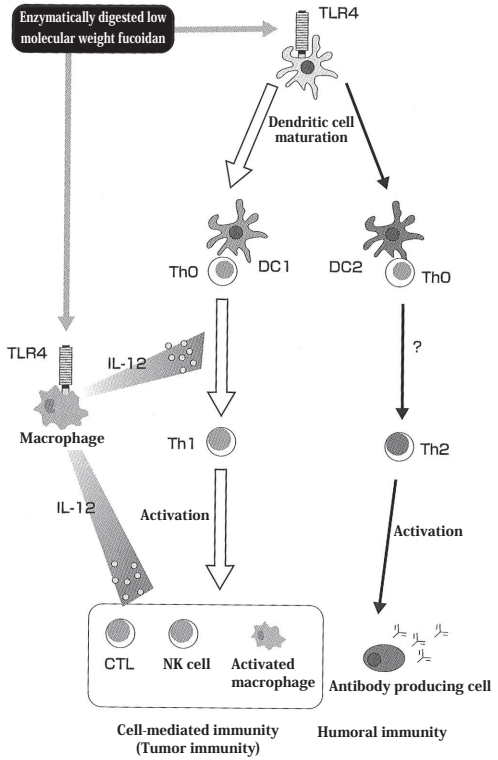
However, the risk factors increase by smoking habit, imbalanced eating habits, lack of exercise, etc., while the antagonizing anti-oncogenes also gradually destroyed.

Moreover, the increase in the reactive oxygen species by a superfluous stress also reduces people's immune strength, and is considered to be the major cause of cancer development.

Under a condition that the defense system called immune strength has reduced, cell growth will be enhanced endlessly when one normal cell mutated to a malignant cancer cell.

Therefore, it can be said that whether or not the existence of immune strength is closely associated with the generation of a cancer cell, and subsequent multiplication.

Here, the tumor immunity activation mechanism by low molecular weight fucoidan elucidated through research is presented by a diagram.



• Mechanism of tumor immunity activation by enzymatically digested low molecular weight fucoidan.

The macrophage which is one of the immunocytes functions like the patrol captain who looks out for a foreign enemy's invasion. When a polysaccharide like fucoidan is taken into the body, the "antenna" captain bears will be stimulated.

Here, an antenna is scientifically called a receptor (sensor) referred to as Toll-like receptor (TLR) 4 which recognizes the basic structure of sugar. TLR4 stimulated macrophage secretes the substance for attacking a foreign enemy. This substance is IL-12 and is also called anti-tumor cytokine. IL-12 will act to activate immunocytes and let them to secrete protein called interferon γ that can attack cancer cell.

Recent years, it has been realized as a characteristic of macrophage that it will recognize “cancer cells as a foreign substance and tending to eliminate them despite cancer cells are a part of the body”.

Thus, low molecular weight fucoidan has effects to stimulate immunocytes and increases cell numbers followed by the activation of immunocytes in the body. Although the detailed mechanism has not been elucidated yet, an assumption that the “immunocytes in the body detect fucoidan and are misled it as pathogenic bacteria since the polysaccharide contained in fucoidan is resembled to that of bacteria, and the immunocytes are activated” is the most probable mechanism at present.

■ Reactive oxygen species scavenging effect

The word "reactive oxygen species" is popular in these days. Reactive oxygen species mean that it is the oxygen species having extremely high reactivity compared to usual oxygen species.

The causal relationship of this reactive oxygen species and cancer is attracting much attention. It has been considered that the generation of reactive oxygen species is related to the alteration of cancer cell to malignant cell, or reactive oxygen species is a direct cause of cancer.

Fundamentally, reactive oxygen species are important substances in the body for killing viruses, pathogenic bacteria and degrading unnecessary cells and substances. However, overproduction of reactive oxygen species lead to inflammation and gene damages which result in the causes and the aggravation of various diseases such as cancer and diabetes.

Moreover, reactive oxygen species are closely associated with the promotion of aging. It has been shown that a life span will certainly be extended when the reactive oxygen species production is appropriately suppressed in an animal experiment. Although human's cell lives much longer compared with that of other animals, the reason is that it has the mechanisms to scavenge reactive oxygen species with multiple enzymatic and non-enzymatic systems. However, the enzyme does not exist to eliminate hydroxyl radical one of the reactive molecular species of reactive oxygen species. It is necessary to ingest vegetable foods as much as possible, and to compensate antioxidants as countermeasures.

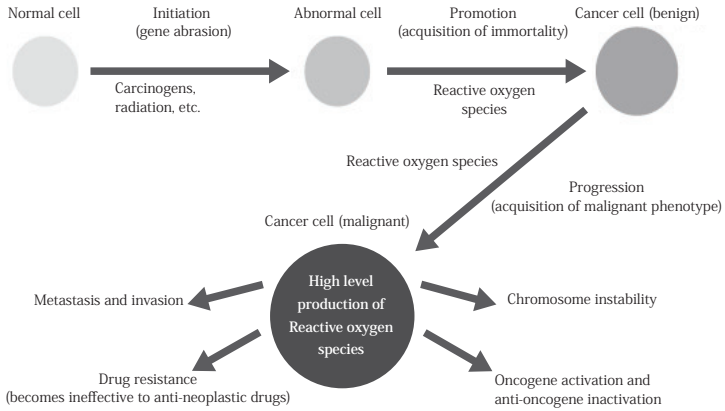
But a point should be realized beforehand is that the suppression of reactive oxygen species production as much as possible is important. Reactive oxygen species will be produced in large amount by strained too much, holding a trouble or accumulating stress. If you feel stresses daily, it seems necessary to look back your way of daily thinking and lifestyle itself.

In the latest research, it has been revealed that an accumulation of hydrogen peroxide by reactive oxygen species is very closely associated with the characteristic of malignant cancer.

The cells bearing the damaged genes are exposed continuously to reactive oxygen species; the cells become immortalized and then soon turned to a malignant cancer cell.

It can be said that the reactive oxygen species are the one can confer the cancer cells more like cancer.

That is, the reactive oxygen species are involved in all events for causing metastasis and invasion for cancer becomes large by the angiogenesis, for an anti-neoplastic drug loses its effect, and for the characteristic rendering the chromosome aberration to occur leading to the activation of the oncogenes and inactivation of tumor suppressor genes.



• Relationship between cancer cells and reactive oxygen species.

It can be expected that the malignant tumor may be reverted to benign tumor when fucoidan scavenge such reactive oxygen species.

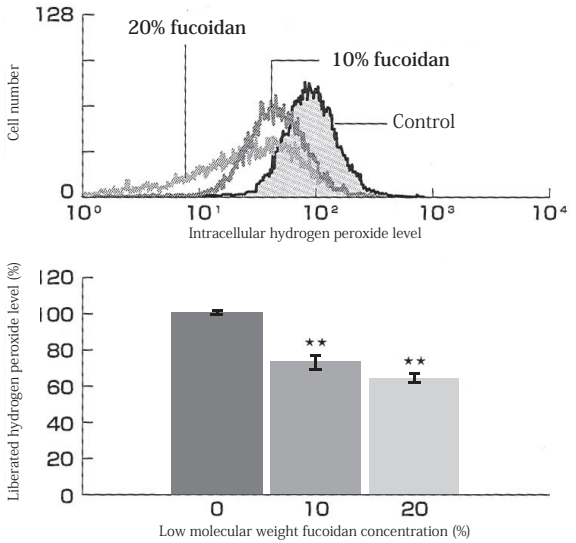
Then, HT1080 fibrosarcoma cells with high metastatic and malignant characteristics were cultured using the culture medium supplemented with 10% and 20% of low molecular weight fucoidan extract.

Although the control (no fucoidan extract) showed the highest peak of reactive oxygen species level, it was evident that reactive oxygen species was scavenged by added low molecular weight fucoidan in dose dependent manner.

Moreover, although a malignant cell secretes hydrogen peroxide to the surrounding cells and gives adverse effect, the cells treated with low molecular weight fucoidan secrete hydrogen peroxide significantly low levels.

When it is clarified that low molecular weight fucoidan functions to scavenge reactive oxygen species, one can expect various cancer phenotypes can be reverted to benign cells.

In regard to cancer metastatic process, a glycoprotein called integrin, and p-secretin are closely involved when cancer cell adheres to the basement membrane as a first step. Although it is clear in many researchers' experiments that low molecular weight fucoidan blocks the cancer cell adhesion to basement membrane, we investigated the effect of the protease (proteolytic enzyme) called MMP (matrix metal protease) secreted at the time of adhesion. Cancer cells become easier to move as MMP degrades proteins such as collagen surrounding the cells. It was found that low molecular weight fucoidan inhibits the gene expression and activation of MMP.



• Scavenging of intracellular reactive oxygen species and suppressive effect of hydrogen peroxide liberation by enzymatically digested low molecular weight fucoidan.
Intracellular hydrogen peroxide level was measured using flow cytometer with DCFH-DA fluorescent dye after exposing enzymatically digested low molecular weight fucoidan to HT1080 cells. In upper figure, higher level of reactive oxygen species is indicated going toward rightward in the X-axis. Y-axis represents cell numbers. The lower figure represents the concentrations of hydrogen peroxide liberated in the medium.

Chapter 3 Presentation in The Japanese Cancer Association and The World Cancer Meeting

■ Combined use of anticancer drugs and low molecular weight fucoidan

Many of anti-neoplastic drugs intend to expose reactive oxygen species to cancer cells and kill them. While, it was explained in the end of the preceding chapter that low molecular weight fucoidan has the reactive oxygen species scavenging activity. This means that there is a doubt about whether the effect of an anti-neoplastic drug is weakened by neutralizing each other when an anti-neoplastic drug and fucoidan are used together. However, the reports are accumulated by the doctors who are using low molecular weight fucoidan that "a curative effect will increase markedly when an anti-neoplastic drug and fucoidan are used together". Let's introduce the latest clinical cases.

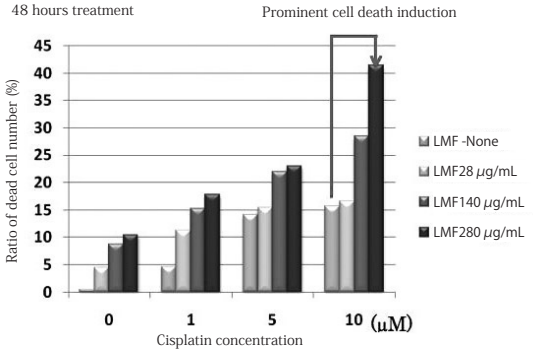
In Spring of 2009, a 76-year-old male is diagnosed as lung cancer and treatment was started from July with antineoplastic drug (taxol). As the side effects were rather strong due to his age, the family asked for the second opinion for alternative treatments in another hospital in Fukuoka. It was the low molecular weight fucoidan which the doctor recommended. The patient started to drink low molecular weight fucoidan together with an anti-neoplastic drug from the 10^{th} of the same month. When having continued drinking of 300 ml of low molecular weight fucoidan per day, the tumor marker returned to the normal value after two weeks. One month later, image inspection shows the remarkable regression of a 6-cm tumor of initial size, and lull state is maintained as of January 2010.

The medical doctor hypothesized that "a certain action of low molecular weight fucoidan improved the effect of the anti-neoplastic drug" Although a medical based reason is still unknown, as I introduced previously, there was also an immune strength potentiating effect in addition to an apoptosis induction effect in low molecular weight fucoidan. Therefore, many doctors considered such a vague causal relationship as that “the side effects were alleviated and led to improve appetite thereby regained vitality to face against cancer”.

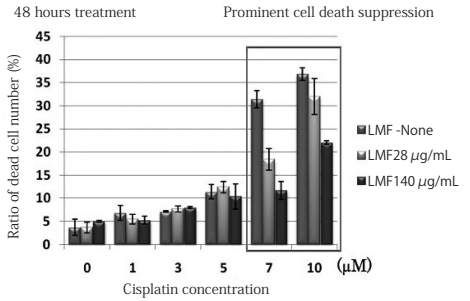
■ Merit of the combined use revealed by experiments

In order to obtain scientific substantiation for the question “Why does the anti-cancer effect reinforce by combined use of an anti-neoplastic drug and low molecular weight fucoidan?”, we carried out two assay methods; the dead cell staining method by trypan blue, and a cell cycle analysis method (sub G1 analysis). The used anti-neoplastic drugs are cisplatin and mitomycin. Cisplatin is the platinum tablet validated for many types of cancers, and has played the central role of the present anti-neoplastic drug as a medical treatment. However, there are intense side effects, and when it is serious, results in a deterioration of kidney function. Moreover, an anti-cancer antibiotic drug, mitomycin exerts its anticancer effect by blocking DNA split, and DNA replication. As side effects, bone marrow suppression is induced more easily compared with other anti-neoplastic drugs, and the cautions are required to infection, or anemia and a bleeding tendency. The renal damage is tended to appear even though many cases result in minor symptoms.

First, the cisplatin and low molecular weight fucoidan both of which with various concentrations were added into the cancer cell (HT1080), and the rate that a cancer cell starts apoptosis was investigated. Then, 2 times or more higher apoptosis induction effect were found compared with low molecular weight fucoidan untreated cell.



• Enhancing effect of anti-neoplastic drug induced apoptosis on a cancer cell (HT1080) by low molecular weight fucoidan.
Low molecular weight fucoidan enhanced cisplatin induced cell apoptosis.



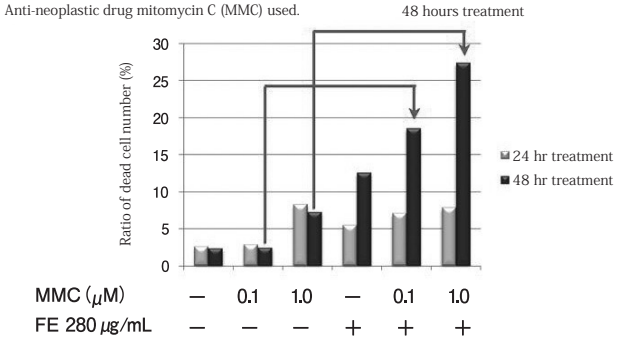
• Suppressive effect of anti-neoplastic drug induced apoptosis on a normal cell (TIG-1) by low molecular weight fucoidan.
Low molecular weight fucoidan extract suppressed cisplatin induced cell apoptosis.

On the other hand, the apoptosis induction of healthy cells (TIG-1) is suppressed.

That is, low molecular weight fucoidan enhanced the apoptosis of the cancer cell by anti-neoplastic drug cisplatin, and it has been observed that there is a protecting effect for the normal cells.

Moreover, the same result is obtained when the combined use of mitomycin and low molecular weight fucoidan is applied to the cells.

The greatest difficulty using an anti-neoplastic drug is it gives damage even to a normal cell, and causes intense side effects.



• Enhancing effect of anti-neoplastic drug induced apoptosis on a cancer cell (HT1080) by low molecular weight fucoidan (2).
Low molecular weight fucoidan enhances mitomycin C induced cell apoptosis.

Some patients suffer from a severe damage both in mind and body due to nausea, loss of appetite, depilation, etc. However, the quantity of the anti-neoplastic drug in use can be reduced as the use of low molecular weight fucoidan together enhances an apoptosis induction effect. In this way, it is thought that the damage will be mitigated for both in mind and body

The doctors of contemporary medicine have been wished for "if there is such an anti-neoplastic drug" that can acts only on a cancer cell, no intense side effects, and does not damage immune strength. I think it will be expanded that the foundation for low molecular weight fucoidan is widely acknowledged and accepted by the doctors who only opened their eyes on using anti-neoplastic drugs.

In order to fight against the cancer as a severe illness, it is necessary to elaborate all approaches without adhering to a fixed idea. The idea of carrying out the medical treatment and the care by incorporating various alternative medicines including western and oriental medicines and make use of the advantageous points inherent to each medicine is "integrative medicine". Isn't the synergistic effect by an anti-neoplastic drug and low molecular weight fucoidan truly a value of integrative medicine? I continue to advance the fucoidan research and wish to establish a concrete therapeutic discipline.

■ Low molecular weight fucoidan distinguishes normal cells from a cancer cell

It is difficult that the anti-neoplastic drug developed as a result of various researches to distinguish normal cells from a cancer cell. However, low molecular weight fucoidan derived from seaweed acts only on a cancer cell. I would like to explain this unique mechanism.

Although it becomes somewhat technical to explain, we have reported this mechanism at the 69th Japanese Cancer Association scientific general meeting in September 2010.

What happens in the process in which a normal cell changes to a cancer cell? Changes take place in a sugar chain synthetic pathway.

A sugar chain is like the network which connects each cell.

Front entrance in the 69th Japanese Cancer Association scientific general meeting venue.

Since the structure of a surface sugar chain changes during transformation process to a cancer cell, the binding affinity for Concanavalin A (ConA) which is a kind of lectin capable of recognizing sugar chain becomes increased. This is known for many years.

We found that the apoptotic cancer cell death was enhanced by fucoidan treatment accompanied by the degree of the ConA binding affinity of many cancer cells increased.

When the action mechanism was investigated, it turned out that the gene expression of N-acetyl glucosamine transferase (GnT-V), one of the enzymes commonly upregulated in the process of oncogenesis is notably suppressed by fucoidan. Moreover, the gene expression of regulatory protein Ets-1 which regulates the gene expression of GnT-V was also suppressed notably.

Thus, GnT-V and Ets-1 attracts attention as they are closely involved with the malignant characteristics as metastasis, invasion and others.

It seems that low molecular weight fucoidan suppresses an unusual sugar chain synthetic pathway of a cancer cell causing a surface sugar chain alteration which gives likeliness to start apoptosis.

It has been known that the fucose contained in low molecular weight fucoidan has an important role to maintain normal functions of surface sugar chain and to maintain the tumor immunity for killing a cancer cell specifically. In normal healthy cells, the gene expression of GnT-V and Ets-1 was basically suppressed in low level and was not observed a noticeable change even after low molecular weight fucoidan treatment.

Although it is scientifically very difficult to distinguish the differences between a cancer cell and normal cells, low molecular weight fucoidan can recognize a cell and exert its selectivity for identifying transformed cells only and lead them to apoptosis.

Chapter 4 Perspectives on Future Research

■ Effectiveness ratio of low molecular weight fucoidan shown by clinical cases

At present, one out of three Japanese has passed away due to cancer. It is said that one out of two persons will develop cancer soon. It is thought that one of the factors is one's eating habits. Originally, Japanese eating habits were mainly vegetable fat, such as vegetables and soybeans constituting a balanced diet. However, the intake of animal fat was increased by westernized diets, and additives such as the colorant and antiseptic, and the chemically synthesized agricultural pesticides and the manure have come to be used along with mass consumption of foods. We are now ingesting such chemical substances constantly.

Moreover, the increase in the reactive oxygen species by a superfluous stress also reduces people's immune strength, and is thought to be the major cause of cancer development.

Contemporary medicine has been advanced by developing sovereign remedies against the foreign invasive diseases and copes with excising the infected portion. However, there is no way to treat the diseases which cannot identify the causes of a specific disease or the diseases which changes unpredictable manners. Many of the present day illness including cancer with difficult to treat are in connection with the essential nature of a life system, and it is because the life system itself has not been completely understood as yet by today's science.

Although fucoidan extracted from the living organisms called mozuku seaweed is expected as a way to countermeasure against a modern disease, there are many unidentified aspects and research has just started. The researcher of many countries in the world is working in this area, and it is still in the middle of an elucidation.


In the laboratory of Kyushu university

However, the clear result has been already obtained as a standpoint of clinical trials. For example, the fucoidan treatment was started since March 2003 in the hospital located at Wakayama Prefecture.

About 80% of the 82 patients (including 47 terminal patients) who ingested low molecular weight fucoidan showed improvement of their conditions. The effectiveness ratio of about 80% obtained by fucoidan is a remarkable numerical value among other various supplements. Moreover, many clinical cases have shown that the enhancement of tumor immunity and improved QOL (quality of life), the life span extension, a healing effect, etc. are obtained by drinking low molecular weight fucoidan.

Even though such positive results are in our hands, it will take many more years elucidating a complete mechanism in terms of medical standpoint of view. In the following chapter, we would like to describe the objectives that we would like to solve as immediately as possible in the future.

■ **Toward identification of effective agents to induce cancer-specific cell death**

In chapter 2, I introduced the 3 major effects of low molecular weight fucoidan to a cancer cell. Although these effects are clarified both at clinically and experimentally in the laboratory, the active ingredients or action mechanism are not fully elucidated.

Presently, what we pay our attention to clarify is an ingredient having apoptosis-inducing activity of low molecular weight fucoidan. When we can narrow down these ingredients to a certain extent or identify the ingredients leading a cancer cell to a natural death, these substances bring the cancer cells to apoptosis many fold effectively and reduce the patient's burden greatly.

In the experiment, fucoidan was first extracted twice with 70% ethanol. Then, the fraction of the high molecular weight fucoidan was precipitated and the apoptosis inducing activity of supernatant portion was increased. When the supernatant portion was evaluated using the different equipment, higher activity was detected in the relatively low molecular weight fraction.

Next, we tried to separate active ingredients depending on the ionicity using a various kinds of ion exchange chromatography. With this method, active ingredients were attracted mostly to SP FF of a cation exchange chromatography. We succeeded to obtain a fraction with a very high activity when fractionated by means of a molecular weight using a reversed phase chromatography.

From the above result, it is thought that the apoptosis inducible active ingredient is contained large quantity in a low molecular weight fraction with high cationic polarity. We are at the stage to be able to specify the active ingredients when apply this fraction to mass spectrometric analysis.

Next, we attempted to elucidate the mechanism which low molecular weight fucoidan leads a cancer cell to a natural death. When we find the action mechanism and prove it scientifically, more doctors and patients feel easy to use low molecular weight fucoidan.

As a result of the experiment using leukemic cell line HL-60, low molecular weight fucoidan was found to suggest a higher possibility that it induces apoptosis by stimulating not only death signal pathways but also survival ones. Moreover, the possibilities were suggested that fucoidan participates in an apoptosis induction by phosphorylating JNK and p38 acting as intracellular signaling factors and by suppressing oncogene Bcl-2.

Thus, low molecular weight fucoidan gives all possible stimuli to a cancer cells and lead them to a natural cell death. In conclusion, there is no doubt that fucoidan is a functional food with a very high apoptosis induction effect and it will attract more people's attention hereafter. When we confirm the similar effects in the cell lines other than HL-60 in the future using the identified active substances, we will be able to prove that fucoidan effectively induces apoptosis in various cancers.

Chapter 5 In Order to Overcome Cancer

■ **Suggestions made by Karolinska Institute**

I was born in Amami Oshima and have continued research on the antitumor substances for many years in Kyushu University. I thought that the drinking water and foodstuffs containing strong antioxidative substances will contribute to prevent cancer development and treatment; in particular I have had an interest in electrochemically reduced water containing active hydrogen in large quantities. I have received many reports that "when electrochemically reduced water is consumed 4-6 liters per day, cancer will disappear." However, even if the validity is known on the basic research level, it is not easy to find the mechanism of how it acts in the body.

Moreover, it is difficult to drink a lot of electrochemically reduced water every day for the physically weakened patients and thus the difficulty remained in clinical application. I have been continuing to study hoping to find the best substance exerting useful effects in a small quantity without side effect. At such time, there was the unbelievable report which mentioned in the beginning of this book that “cancer disappeared when ingested fucoidan”.

Presently in Japan, majority of doctors declare that "cancer will not disappear by ingesting foods or water". Even when cancer disappears by a supplement actually, doctors seldom show interest.

I myself thought that it has a great possibility as a supplement which antagonizes cancer while advancing research of low molecular weight fucoidan.

The clinical examples of cancer improvement by low molecular weight fucoidan are preceded rather than scientific research. Only the result has come out first, even though action mechanism has not been elucidated. The doctors who believe in contemporary medicine, it will be referred to as "one of the fake alternative medicine." Those patients as well as the doctors who aspire to make use an integrative medicine also feel impatient. It was considered to be a very significant work to advance low molecular weight fucoidan research for such people.

In 2008, I invited a scientist in the toxicology laboratory of Karolinska Institute, Sweden to Kyushu University. Karolinska Institute is a research institution of the world's best standards in the field of biomedicine, and is an authoritative research institute which has a selection committee of the Nobel Prize for physiology and medicine. I discussed with the scientist of such research institute about the anticancer efficacy of low molecular weight fucoidan. Then, the scientist suggested that there might be a possibility to compose an excellent paper when the mechanism is clarified because they never heard such effect that "fucoidan induces apoptosis by acting only on the surface sugar chain of cancer cell without affecting normal cells”.

Moreover, I was invited by Karolinska Institute on the 12th of May in 2011and presented the results of electrochemically reduced water and low molecular weight fucoidan studies in the Research Institute of Environmental Medicine. Emeritus Professor Sten Orreniius who has acted as a member of the Nobel prize candidate's for physiology and medicine selection committee for years commented after my presentation that "it is valuable that the research in electrochemically reduced water progressed very much compared with four-years ago, and fucoidan research also is very important" from a viewpoint of the realistic medical treatment of cancer.

Incidentally, I made a presentation about the apoptosis induction effect of low molecular weight fucoidan, etc. in the functionality laboratory of the agricultural food research institute at Research Institute Chinese Academy of Agricultural Sciences in Beijing, on the 9th of the same month. Researchers had interest in my presentation and commented that "If there is an opportunity, I would like to carry out joint research." On the 15th of the same month, I attended in “The European Society for Animal Cell Technology meeting 2011 convention" held in Vienna, Austria, and presented an apoptosis induction mechanism. On the 23rd of the same month, research presentation was made in the "4th World Cancer Meeting" held in Dalian, China under the title of " the selective cancer cell death and an induction of sugar chain synthetic pathway modulation by an enzymatically digested low molecular weight fucoidan extract". After this presentation, an American scientist made a comment that the research is very interesting and offered me to write a review in the Journal he is involved.

A presentation scene in the "4th World Cancer Meeting" (Dalian, China)

Various researchers in the world including myself are promoting the research paying attention to the anticancer efficacy of low molecular weight fucoidan.

Illness of the "cancer" as currently considered to be impregnability is finally started to see the clue to unravel the hidden mechanism.

Although, it is not easy to find the way to get to the summit, I think that we now finally reached to the half-point from the top.

■ The most important issue is to choose the best and convinced treatment

The term Illness in Japanese can be interpreted as an illness of mind, and is deeply related with spiritual strength. Illness may become better or may worsen depending on how to maintain the mind. Isn't medicinal effect probably different even if they are taking the same medicine by those who think that "such a medicine cannot be effective", and by those who think that "this medicine must be absolutely effective?

Although living with a belief is important for man, I think that the belief should not be either stubbornness or persistent. For example, there may be many people who think that only western medicine is worth to trust and think that the integrative medicine incorporating dietary therapy, eastern medicine, a supplement treatment, etc. is not worth to believe. However, isn't it necessary to have pliability in selecting the best treatment according to the condition at that time instead of just simply comparing these methods?

There is an enough possibility that low molecular weight fucoidan or other functional foods and functional water could also coexist with the present western medicine when their validity is proved by the future research. However, whether such alternative medicine is accepted or not at present depends on each doctor's judgment and the patient own judgment.

When diagnosed as cancer, or when life expectancy is pronounced, it is also one view to accept the diagnosis silently and solemnly. However, there is also a view of trying to find the next action without giving up. I think that it is also necessary to ask a family doctor once again about the treatment until you are fully convinced in the future direction. Moreover, it is a way to take a step by expanding a view to face this situation by consulting with the doctor who practices integrative medicine.

Paying attention to the anticancer efficacy of low molecular weight fucoidan which I study, the doctors who practice integrative medicine using low molecular weight fucoidan as one of the supplement treatments are increasing in number all over in Japan. Such doctors are working every day in order to contribute to promotion of the research covering "cancer therapy at large, recurrence prevention, etc." in integrative medicine, and improvement in suitable medical treatment by having the investigative commission and study group of clinical cases sharing clear information and knowledge about low molecular weight fucoidan, etc.

A scene in a tumor board review meeting

Doctors are also fighting desperately with cancer. A hope will not come by petrified with uneasy feeling or being lost. I think that we should go one step forward with courage under any circumstances.

● Author affiliation

Sanetaka Shirahata

In 1978, he graduated Department of Food Science & Technology, Graduate School of agriculture Kyushu University. Doctor of Agriculture degree was granted on completion of doctoral course in agriculture. In 1987, he studied as a visiting associate professor in the Department of Biochemistry and Biophysics in Oregon State University, USA. In 1989, he is appointed as an associate professor of Kyushu University. In 1995, he is appointed as a professor of the Laboratory of Cellular Regulation Technology, Graduate School of Genetic Resources Technology, Kyushu University. In 2003, he is appointed as a joint professor of Division of Life Engineering, Graduate School of Systems Life Sciences, Kyushu University.