IJRD International Journal of Research & Development Organisation

ISSN: 2455-7676

Journal of Biological Science

Prevention of swelling of liver tumor in Sprague-Dawley rat fed with the Kinu-Mozuku, an edible Japanese brown alga grown at seashores in Noto-peninsula.

Kou Hayakawa 1*, Takeaki Nagamine 2.

1 (Retired) Department of Endocrinology and Metabolism, National Research Institute for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan

2(Retired) Graduate School of Health Sciences, Gunma University 3-39-15 Showa-machi, Maebashi, Gunma 371-8514, Japan

* To whom correspondence should be addressed.

2-5-B-308 Fukiage-fujimi, Kohnosu-City, Saitama 369-0121, Japan

Tel; +81-48-548-1733; Fax; +81-48-548-1733

e-mail: amokha123@true.ocn.ne.jp

Dedication: In memory of the encouragement of my beloved daughter Reiko Hayakawa (21 November 1979 – 1 February 2007).

Abstract

Aim: Swelling of the tumor in the liver is difficult to stop by ordinary medicine. Then, new and safe drug preventing tumor-growth is required.

Method: Three Sprague-Dawley rats, injected chemical carcinogen of diethyl- nitrosamine for 12 weeks, are then fed the edible brown-alga Kinu-Mozuku, grown at seashores around Noto peninsula, Japan, for 3 weeks.

Results: No liver tumor is found in three Kinu-Mozuku-fed sample rat. On the contrary, all the three control-rats, fed without Mozuku, show very large tumor in their livers. This observation is statistically significant (P = 0.05 by Mann-Whitney's U test).

Conclusion: Japanese edible Kinu-Mozuku (Nemacystis decipiens) is proven to be the strong foodstuff/drug preventing enlargement of liver-tumor in the outbred rat. Thus, the edible fucoidan is expected to be the powerful and safe drug/medicine preventing and healing the human HCC and also the other intractable human cancers.

Keyword; Kinu-Mozuku, brown algae, fucoidan, Sprague-Dawley (SD) rat, outbred, liver tumor, HCC, diethylnitrosamine (DEN).

1. Introduction

Both biotin (vitamin H) and lipoic acid (thioctic acid) are indispensable growth factors (vitamins) for the adult humans. Lipoic acid (LA) has recently been re- cognized as a vitamin for human cells of cultured-hepatocarcinoma HuH-7 and urinary-bladder-cancer T24, but cultured-cell lines derived from the rat seem to biosynthesize LA; i.e., LA is biosynthesized and cytotoxic to TRL1215 liver-cells of Fischer F344 rat and to NBT-T2 bladder-cells of Rattus norvegiscus (unpublished observation). Biotin and LA are originally discovered from duck's egg-yolk (Kögl & Tönnis, 1936) and from potato (Snell, Strong, & Peterson, 1937), respectively. Both of these vitamins is not biosynthesized in the adult humans possibly due to the strong repression of genes for biotin and LA synthetases after the parturition of humans. Biotin is an essential coenzyme for elongating acyl-chain of carboxylic acids via its specific binding ability to the carbon dioxide (CO2) (Lynen, 1964). LA is also essen-

tial coenzyme for NADH-production and providing energy to human cells; i.e., producing acetyl-CoA from pyruvic acid by pyruvate dehydrogenase (PDH), and NADH (reduced-form nicotinamide adenine dinucleotide) through the respiration system in citric acid cycle (Stanley Tsai, Burgett, & Reed, 1973).

Thiol-type biotinidase/lipoamidase (formerly designated separately as biotinidase (E.C. 3.5.1.12) and lipoamidase (E.C. 3.5. -. -.)) is an important enzyme in humans by recycling

biotin and LA (Hayakawa & Oizumi, 1988 a and b; Oizumi & Hayakawa, 1989; Nilsson & Kågedal, 1993; Hayakawa, Hirano, Oizumi, & Hosoya,1998). Interestingly, we recently have found that avidin in hen's egg-white is the biotin/LA-binding protein; i.e., D, L-LA shows a stronger affinity than D-biotin to avidin (Hayakawa et al., 2007). Previously, we have compared the biotinidase/ lipoamidase kinetics between normal and cancer tissues in 14 human livers, and have found that Kip (inhibition constant by the product biotin) is significantly ($P < 0.01$; Mann-Whitney's U test; two-tailed test) increased in the cancer tissues as compared to the normal tissues (Hayakawa & Nagamine, 2009). The augmented Kip value is recognized as the result of increased free-form biotin in the cancer tissues (Hayakawa & Nagamine, 2009). Human serum biotinidase/lipoamidase is a glycoprotein enzyme (Chauhan & Dakshinamurti,1986; Hayakawa, Yoshikawa, & Watanabe, 1994), and N-acetyl neuraminic acid (NANA) within its N-linked glycochain is found to enhance the affinity (Amo) for the biotin-amide substrate (unpublished observation). This glycoprotein-enzyme also contains the L-fucose within its glycochain (Hayakawa, Yoshikawa, & Watanabe, 1994), although precise role of L-fucose residue to enzyme kinetics has not yet been precisely determined. Therefore, we have searched the polysaccharide possessing the ability to calm the exasperated enzyme-kinetic of Kip, and have discovered that fucoidan (fucan; sulphated poly-fucose containing D-glucuronic acid) from Okinawa-Mozuku (Cladosiphon okamuranus)is significantly able to decrease Kip in the HCC supernatant (poorly differentiated type) and inversely increase Kip in the normal LC supernatant in vitro (Hayakawa & Nagamine, 2009).

Fucoidan and/or fucan (original name; Fukoidin) has been firstly discovered from the inedible brown algae such as Fucus vesiculosus (Kylin, 1915), and various physiological, biochemical, and glycol-biological effects of this sulphated poly-fucose have been described (Berteau & Mulloy, 2003). Therefore, it is surely reasonable that the Okinawa-fucan has changed the enzyme-kinetic of Kip possibly through the interactions

between polysaccharide-chain of fucan and N-type glycochains (un- differentiated type) of human liver biotinidase (Hayakawa & Nagamine, 2009).

Furthermore, growth and proteomic studies using hepatocarcinoma cell-lines

indicate that the fucoidan derived from the edible Ishi-Mozuku (Stony-Mozuku; Sphaerotrichia divaricate; product of the seashores of Noto peninsula, Ishikawa, Japan)

retards the growth of the well-differentiated-type cell-lines of HepG2 and HuH-7 at 0.102 mg/mL, but does not retard the growth rate of the fetal-undifferentiated hepatocyte Hc cells. The state of differentiation is assessed by using proteomics; i.e., well-differentiated-type liver cell of HepG2 expresses albumin and ceruloplasmin, but

does not express α-fetoprotein and fetal-type biotinidase, however undifferentiated-type liver cell of Hc expresses in a reversed manner in the gene expressions (Hayakawa & Nagamine, 2014). Growth study under the microscope also shows that the fucan from Okinawa-Mozuku retards the growth of pediatric hepatoblastoma HuH-6 cells, but does not retard the adults' hepatoma cells HepG2 and HuH-7 (unpublished observation). These results suggest that fucan derived from the Okinawa-Mozuku is effective to the

undifferentiated-type cancer (pediatric cancer). Both Japanese edible Ishi-Mozuku fucoidan and Okinawa-Mozuku fucan shows no effect on the growth of normal fetal-hepatocyte cells (Hc cells), but inedible Ireland's fucoidan shows the immediate cytotoxic effect (Hayakawa & Nagamine, 2014).

Further, it is recently found that fucan derived from Okinawa-Mozuku is actively transported across the human intestinal cell line, Caco-2 (Nagamine, Hayakawa, Nakazato, & Iha, 2015). This finding strongly suggests that the oral administration method of edible fucoidan and Mozuku is effective in healing the cancers in the rat and in the humans.

In this report, it is presented that a novel and wonderful outcome by the Kinu-Mozuku (Silky-Mozuku; Nemacystis decipiens; product of Noto peninsula, Ishikawa, Japan) is significantly

able to prevent the enlargement of the tumor in chemically carcinogen (DEN)-treated livers in the Sprague-Dawley (SD) rat.

2. Materials and Methods

2.1 Chemicals and reagents

Inedible fucoidan (derived from a brown alga of Fucus vesiculosus; product of Ireland) and carcinogen of diethylnitrosamine (DEN) were purchased from Sigma (St. Louis, MO, USA). Polyethylene-glycol 1000 monocetylether (PGC) was from Nacalai Tesque (Kyoto, Japan). Fetal calf serum (FCS) was from Moregate BioTech (Bulimba, Australia). Edible Japanese-foodstuffs (brown algae) of the Kinu- Mozuku (Silky-Mozuku; Nemacystis decipiens) and the Ishi-Mozuku (Stony-Mozuku; Sphaerotrichia divaricata) were both purchased from Koh-Sushi Co. Anamizu-machi, Hohsu-gun, Ishikawa, Japan). Edible Okinawa-Mozuku (Futo-Mozuku; Thick- Mozuku; Cladosiphon Okamuranus) of Main-island of Okinawa, Japan, was donated from the Graduate School of Health Sciences, Gunma University (Maebashi, Gunma, Japan). Other Japanese edible brown-algal foodstuffs of Mekabu (sporophylls of Wakame (Undaria pinnatifida); Ishinomaki, Miyagi), Kombu (sea tangle of Laminaria japonica, Ofunato, Iwate), and Iwa-Mozukus (Rocky-Mozukus; Tinocladia crassa) from seashores of the Japan Sea such as Sadogashima Island (Niigata), Shimonoseki- city (Yamaguchi), and Fukaura-machi (Aomori) were purchased from grocery stores.

2.2 Preparation of fucoidan from the Silky-Mozuku

Silky-Mozuku (7 kg in wet weight) was extensively desalted with water, and dried under sunlight, and then fanned by an oven at 75 ℃. Dried Mozuku was then powdered by using a pestle and mortar. Powdered Mozuku (195 g of dry weight) was then mixed with 10 kg of ordinary feed for rat (2% w/w), and sterilized by gamma-ray radiation at 10 kGy (Clea Japan Inc., Tokyo, Japan). Sterilization was done by way of caution,

since Silky-Mozuku was rich in the nutrition of biotin (Hayakawa et al., 2009).

Crude fucoidan was prepared from the Silky-Mozuku by extracting with San-bai-zu (mixture of Yone-zu (vinegar containing 4% acetic acid derived from rice) : Soy sauce (thin sauce made from fermented soy beans) : sugar = 3 : 1 : 1 w/w/w), and stood for overnight at 4 ℃. Then, the supernatant crude-fucoidan fraction was washed by adding ethanol to become 80% (v/v). Aggregated white-filamentous fucoidan was collected by filtration, and this washing with 80% ethanol was repeated thrice. Extracted crude fucoidan was then dried with fan oven at 75 ℃. Dried fucoidan (51.0 mg) was dissolved in 1.0 mL of water, and sterilized by autoclave (120 ℃, 20 min), and was used in the experiment with the human liver-cell lines.

2.3 Experiment using the Sprague-Dawley (SD) rats

SD rats at 7-months of age (male, outbred strain, closed colony) were purchased from Clea Japan Inc. (Tokyo, Japan). LEW rats (8-months of age, male; inbred strain) were also purchased from Sankyo Labo Service Co. (Tokyo, Japan). Rats were kept in the animal facility of National Institute of Child Health and Development, with a 12-h light/dark regime. They were fed individually in the separate cages in order to prevent the hierarchy phenomenon during food intakes. In order not to be infected by the microbes, the cages of the rat were set in the sterile room. At the age of 8-months, the rats were intraperitoneally injected 50 mg/kg of DEN once a week according to the protocol (Schiffer et al., 2005). After 12 weeks (3 months) of injections, the rats were separated into two groups, and the control rats (C1, C2, C3) and the sample rats (S1, S2, S3) were then fed with ordinary (without Mozuku) and Mozuku-added (16 mg of dry weight/g of dry feed; 1.6 %) feeds, respectively. Oral administration of Silky-Mozuku together with DEN injection was continued for three weeks in the sample rats, and the usual feeding and DEN injection was also continued in the control rats.

Both groups of rats were fed with ordinary feed for additional one week to recover from the acute injury of the livers. After 16 weeks (4 months) of the experiment, all the rats were killed to compare the livers between two groups of rats. There were no significant differences in the amount of food-intake and the volume of water-intake per day, and also in the body weights between two groups during the experiment. Photographs were kindly taken by the professional photographer of the National Center for Child Health and Development. All the protocols were carried out in accordance with ethical guidelines for laboratory animals of National Research Institute for Child Health and Development.

2.4 Experiments using the cultured liver-cell-lines

Human hepatoblastoma HuH-6 (from 1 year old, Japanese male baby), hepato-carcinoma HuH-7 (from 57 years old, Japanese male), and HepG2 (15 years old, male of USA, Caucasian) were purchased from Rikagaku Kenkyusho (Tsukuba, Ibaragi, Japan). Normal human fetal hepatocyte Hc cells (obtained from USA) were kindly donated from the Graduate School of Health Sciences, Gunma University. Cell culture of HuH-7 was performed on usual non-coated plastic plates, and other cells were cultured on the collagen-coated plates. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, streptomycin (0.1 mg/mL) and kanamycin (0.5 mg/mL) in a humidified 37˚C/5% CO2 incubator. Sterilized fucoidan solution was added to the culture medium at a concentration of 0.102 or 0.200 mg/mL.

2.5 Biochemical analysis

The proteomics protein-direct-microsequencing-decipering (PDMD) method was performed on the liver cell-lines as described (Hayakawa & Nagamine, 2014; Abe, Hayakawa, Ihara, Deguchi, & Nagamine, 2015).

HPLC protein determination with size-exclusion chromatography (SEC) was performed as described previously (Hayakawa et al., 2001).

2.6 Statistical analysis

Since the distribution patterns in biology and biochemistry showed no Gaussian distribution (Gauß-Verteilung), a non-parametric method of Mann-Whitney's U test was performed. It was concluded that P≦0.05 (one-tailed test; n1 = 3, n2 = 3; all the three values in one group was considered to have the same rank) between the two groups was significant. Non-parametric statistical analysis was performed as described previously (Terentyeva et al., 1997). Another mathematical consideration and calculation using Bernoulli trials was also performed (Snedecor & Cochran, 1967).

3. Results

In the previous paper (Hayakawa & Nagamine, 2009), we described that fucan from Okinawa-Mozuku (Cladosiphon okamuranus) and heparin reduced the Kip value of biotinidase in homogenates of HCC tissues of poorly differentiated type. Homogenate of hepatoblastoma HuH-6 cells cultured with fucan (0.200 mg/mL) for two days also showed decreased Kip (Hayakawa & Nagamine, 2009). However, we did not adopt heparin due to possible injury on human body (Hayakawa & Nagamine, 2009). Furthermore, inedible fucoidan from Ireland's brown alga (Fucus vesiculosus) at 0.200 mg/mL killed and detached the Hc cells from the plastic plate in 3 days suggesting the presence of strong side effect (Hayakawa & Nagamine, 2014). Therefore, we also did not adopt this inedible Ireland's fucoidan (Hayakawa & Nagamine, 2014). The qualitative microscope examinations indicated that fucoidan from Ishi-Mozuku (Sphaerotrichia divaricata) of Noto peninsula inhibited the growth of HuH-7 and Hep-

G2 (both adult origin and well differentiated type) strongly, but fucan from Okinawa- Mozuku did not at all at the concentration of 0.102 mg/mL (Hayakawa & Nagamine, 2014). Therefore,

other edible Japanese-fucoidans made from Mekabu, Kombu, Iwa-Mozukus (Tinocladia crassa), Stony-Mozuku, and Silky-Mozuku were also tested, and we found that the edible fucoidan derived from the Silky-Mozuku (Nemacystis decipiens) and the Ishi-Mozuku (Sphaerotrichia divaricata) showed the most strong growth-retardation effect on the human hepatocarcinoma cell-lines (Table 1).

Based upon these fundamental results of edible Japanese-fucoidans onto the cultured liver-cell lines, the prevention effect of dried Silky-Mozuku from the growth of liver tumor in the SD rat, which was chemically treated beforehand with diethylnitrosamine (DEN) (Schiffer et al., 2005), was then studied. The outbred Sprague-Dawley (SD) rat, which possessed the normal immunity, was chosen for this study. On the other hand, the use of about 30 LEW rats (inbred strain) with low immunity was in vain (data not shown).

The clear result of the prevention experiment using SD rat was shown in Fig. 1. Six livers in the left panel were seen from the abdominal side, and six livers in the right panel were also seen from the back side. As shown in Fig. 1 (upper part), two control livers without Silky-Mozuku clearly showed the presence of large and developed tumors (C1 and C2). The liver of the right position (C3) unexpectedly possessed the large tumor within the inner portion after the transverse incision (data not shown).

Therefore, all the three control livers (C1, C2, and C3) possessed a large tumor (larger than 15 mm in diameter). On the contrary, all sample livers fed with Silky-Mozuku (S1, S2, and S3) possessed no tumor (Fig. 1, lower line). Statistical significance was calculated by using the Mann-Whitney's U test, and was found to be significant ($P = 0.05$, one-tailed test). Another statistic coin-flipping consideration (Bernoulli trials) also indicated the similar conclusion; i.e., the chance of occurrence of three prevented cases by Mozuku-feeding and three HCC-induced cases was calculated as $1 / 26$ or $1 / 64 = 0.0156$ (significance; $P < 0.02$). Therefore, the foodstuff Silky-Mozuku

showed the preventing effect against the tumor formation and enlargement in SD rat.

4. Discussion

Retardation of cell-growth by fucoidan is assessed by the microscopic examination, and we have found that fucoidan from the Mozuku of Noto peninsula inhibits the growth of hepatocarcinoma cells of HuH-7 and HepG2. Although the growth inhibition effect of mercuric ions on bacteria has been monitored turbidimetrically using nephelometer (Hayakawa, Kusaka, & Fukui, 1975), the growth test of cultured liver-cells is not possible by turbidimetrical method due to attachment of the cells onto the plastic plates as epithelial-like morphology. Therefore, comparison of the wet volumes has been performed after harvesting the cells by the cell scraper, and it is shown that fucoidan (0.102 mg/mL) can retard the growth of HepG2 cells for 2-fold in three-days (Hayakawa & Nagamine, 2014). The MTT (yellow tetrazole-dye) assay method for living-cell analysis gives the apparently numerical-data and has been widely employed in the growth test in cultured cells (Cory, Owen, Barltrop, & Cory, 1991), it is recently found that the similar and parallel results can be obtainable between the microscopic investigation and the MTT assay using bladder-cancer cell-lines (unpublished observation). Furthermore, the coefficients of variation (CV; (standard deviation (SD) / average) x 100 (%)) of MTT assay is very high (usually reaching to about 50%) due to wide-ranges in cellular activity to uptake MTT dye via the active transport (Nagamine, Hayakawa, Nakazato, & Iha, 2015). Therefore, we have not employed yet this famous MTT assay for the analysis of growth of cultured cell-lines.

Differences in the fucoidan effect of growth-retardation have been widely pursued among the edible Japanese-fucoidans, and the fucoidan from Silky-Mozuku and Ishi-Mozulu (product of Noto peninsula) is found to be the most powerful (Table 1).

Silky-Mozuku grows upon leaves of a brown alga of Hondawara (Sargassum fulvellum) in the winter season (from December to March), and is harvested during April and May. The sea

surface temperature (SST) during winter around the Noto peninsula (at latitude 37 degrees north and longitude 137 degrees east; mean 11.8 ℃) is lower than the SST around the Okinawa (at latitude 26 degrees north and longitude 128 degrees east; mean 22.5 ℃). Therefore, it is recognized that the lower SST during the growth of seaweed seems to be important for exhibiting the strong healing-effect by Mozuku. The difference in the healing effect between Silky-Mozuku (Noto peninsula) and Okinawa-Mozuku (Ryukyu) may be occurred by the differences in chemical structures between fucoidan (pure sulphated poly-fucose) and fucan (sulphated poly-fucose with glucuronic acids). Unexpectedly, the fucoidan derived from Ishi-Mozuku (Noto peninsula) does not retard the growth of the undifferentiated-type hepatoblastoma HuH-6 cells, in spite of fucan from Okinawa-Mozuku inversely retards the growth of it. The different transporting capacities may also be expected among the differentiated-hepatoma cell-lines, since different glycochain structures among fucoidans and fucan may exist. Therefore, fucan of Okinawa-Mozuku is easily taken up to the hepatoblastoma HuH-6 cells, and reduces the concentration of D-aspartic acid (Hayakawa et al., 2005). Thus, the mixture of the foodstuffs of Okinawa-Mozuku and Noto-Mozuku may be the good choice when the state of the differentiation of the cancer tissue is unsettled.

Both Japanese edible fucoidan and fucan shows no effect on the normal fetal liver's Hc cells as assessed by the differences in the cell-growth (by the wet volumes and wet weights), but inedible Ireland's fucoidan killed and detached the Hc cells from the plastic culture plate (Table 1; Hayakawa & Nagamine, 2014). Furthermore, it has already been found in Japan that Japanese people in the Jomon and Yayoi periods (BC 1,000-AD 300) has already taken seaweeds and sea algae; i.e., the pottery or earthenware of Jomon and Yayoi, which is adhered by seaweed and sea algae, has been dug up. Then, Japanese people has long safely eaten the Japanese Mozuku without any descriptions of the side effects. Further, it has also recently been reported that the rats fed with edible-fucoidan of Japan in long-time and in excess amounts

show no symptoms of the side effects (Abe et al., 2009). This experiment also indicates that the Japanese edible-fucoidans and Mozukus are the safe drugs/foodstuffs.

Chemically prepared small-molecular-mass fucan (Mr less than 1,000; from natural fucan (Mr 200,000) derived from Okinawa-Mozuku) unexpectedly exhibits the growth-accelerating effect on the hepatocarcinoma HepG2 and HuH-7 cells at 0.100 mg/mL (Table 1). This finding indicates that the characteristics of the large- molecular-weight of natural fucan or fucoidan is the crucially important point to heal the cancer.

Inbred LEW rat has low immunity against invasions of microbes and has long been employed in the surgical transplantation experiments. Humans is outbred and healthy persons have normal immunity. However, patients of cancer have low immunity, and the interesting methods using immunological antigens have already been applied in Japan; i.e., antigens (1) against the glycochains derived from the human-type tubercle bacilli SSM (special substance of Maruyama; Maruyama's vaccine) (Nagae, 1990), and (2) against glycolipids derived from the own or another cancer-patient's urine (Hasumi's vaccine) (Harris et al., 2008). Although these immunological treatments may be effective in some degrees, essential point of view on the nutrition (such as biotin, lipoic acid, and biotinidase) (Hayakawa & Nagamine, 2009) has not yet been satisfactorily incorporated.

Although biochemical analysis on these six SD-rat livers used in this research has not yet been performed, the possible outcomes by the proteomics of the protein-direct- microsequencing-decipering (PDMD) method on these rat livers are reasonably ex- pected to be essentially similar to the results of liver cell-lines as described; i.e., (1) increase of the membrane compartment (Hayakawa & Nagamine, 2014), (2) decrease of +ss RNA virus proteins (Hayakawa & Nagamine, 2014), (3) decrease of D-aspartic acid (Hayakawa et al., 2005), and (4) changes in the Kip of liver biotinidase/lipoamidase (Hayakawa & Nagamine, 2009). Since mechanism of fucoidan effect is the retardation of tumor growth and is general, the

fucoidan from Silky-Mozuku will be effective on the large tumors (late stage cancer) via decreasing the size of liver tumor and stimulating nutritionally the beneficial growth of surrounding normal liver cirrhosis

(LC) tissues (Hayakawa & Nagamine, 2009). Thus, this unique Silky- Mozuku-feeding therapy is surely expected to be effective generally on the adult human HCC and also on the other various tragic human cancers.

It is also noteworthy that all the six SD rats used in this fucoidan test show the comfortable behavior as if they all suggest this fucoidan is surely the sovereign remedy after the 4 months of the experiment. Further, they seem truly expecting to be published the manuscript of this experiment to me, on the contrary the severe and sad situations is observed before this lucky success when using Okinawa-fucan and about 30 LEW rats in vain.

In conclusion, the Silky-Mozuku (Nemacystis decipiens) is shown to be the strong and safe foodstuff preventing liver cancer in the outbred Sprague-Dawley rat. Thus, fucoidan derived from the edible Japanese Mozuku is expected to be the safe and powerful drug/medicine preventing and healing the human HCC and also the other intractable human cancers.

5. Acknowledgements

This work was supported by the Ministry of Welfare, Labour and Health, Japan. We are grateful to the Former Director Prof. Dr. Tadatoshi Kuratsuji, MD, and the Manager of Laboratory of Animal Studies Dr. Hideki Tsumura, D.V.M., of the National Research Institute for Child Health and Development (Tokyo, Japan) for their kind encouragements for this work. One of the authors (K. Hayakawa) is also very grateful to his wife, Mrs. Keiko Sakai Hayakawa, for her kind and constant help and support during his research life.

6. References

Abe, K., Hayakawa, K., Ihara, K., Deguchi, K., & Nagamine, T. (2015). Changes in the hydrophobic proteins in response to biotin administration in serum of infant patients with alopecia as assessed by the protein micro-sequencing method.

Journal of Microbial & Biochemical Technology, 7, 39-46.

http://dx.doi.org/10.4172/1948-5948.1000179

Abe, N., Kawamoto, H., Miki, Y., Kimura, T., Kasagi, K., Ichikawa, O., … Ikeda, T. (2009). Effects of taking of edible fucoidan for long-time and for excess- amount to the rats. The Proceedings of the Joined Meeting in Okinawa by *Japan Society for Bioscience, Biotechnology, and Agrochemistry, Japan Society of Nutrition and Food Science, and Japan Food Science and Engineering Society*, p 16, 31 October (orally presented in Japanese).

Berteau, O., & Mulloy, B. (2003). Sulfated fucans, fresh perspectives: structures, functions, and biological properties of sulfated fucans and an overview of enzymes active toward this class of polysaccharide. *Glycobiology,* 13, 29-40.

Chauhan, J., & Dakshinamurti, K. (1986). Purification and characterization of human serum biotinidase. *Journal of Biological Chemistry*, 261, 4268-4275.

Cory, A.H., Owen, T.C., Barltrop, J.A., & Cory, J.G.. (1991). Use of an aqueous soluble tetrazolium/formazan assay for cell growth assays in culture. *Cancer Communications*, 3, 207-212.

Harris, K.M., Lenz, P., Hankey, K.G., MacVittie, T., Farese, A., Nakajima, K., … Mann, D.L. (2008). Products of anti-CD3/anti-CD28 activated lymphocytes induce differentiation and maturation of dendritic cells and have adjuvant-like activity *in vitro* and *in vivo*. *Clinical Immunology,* 129, 58-68.

Hayakawa, K., Kusaka, I., & Fukui, S. (1975). Resistance to mercuric chloride in *Pseudomonas K-62*. *Agricultural and Biological Chemistry*, 39, 2171-2179.

Hayakawa, K., & Oizumi, J. (1988 a). Human serum lipoamidase. *Enzyme*, 40, 30-36.

Hayakawa, K., & Oizumi, J. (1988 b). Human serum biotinidase is a thiol-type enzyme. *Journal of Biochemistry*, 103, 773-777.

Hayakawa, K., Yoshikawa, K., & Watanabe, T. (1994). Biotinidase: determinations of enzyme activity, chemical structures such as glycochain structure and amino-

acid sequence, and potential physiological roles of the enzyme and the possibility of biotinidase onto clinical applications. *Vitamins (Kyoto)*, 68, 318-320 (in Japanese).

Hayakawa, K., Hirano, M., Oizumi, J., & Hosoya, M. (1998). Isoelectric focusing of biotinidase and lipoamidase with the addition of non-ionic detergent. *Analytica Chimica Acta*, 372, 281-289.

Hayakawa, K., Yoshinaga, T., Hirano, M., Yoshikawa, K., Katsumata, N., Tanaka, T., &

Nagamine, T. (2001). Protein determination by high-performance gel- permeation chromatography: Applications to human pancreatic juice, human bile and tissue homogenate. *Journal of Chromatography B*, 754, 65-76.

Hayakawa, K., Nagamine, T., Li, X-K., Katsumata, N., Ogata, T., & Tanaka, T. (2005). Affinity chromatographic determination of D-aspartic acid in the liver cell lines. *Trends in Chromatography*, 1, 105-110.

Hayakawa, K., Katsumata, N., Yoshikawa, K., Hirano, M., Ogata, T., Tanaka, T., & Nagamine, T. (2007). Determination of lipoic (thioctic) acid by high-per-

formance affinity chromatography with a trypsin-treated avidin-bound column. *Trends in Chromatography,* 3, 31-42.

Hayakawa, K., Katsumata, N., Abe, K., Hirano, M., Yoshikawa, K., Ogata, T., …

Nagamine, T. (2009). Wide range of biotin (vitamin H) content in foodstuffs and powdered milks as assessed by high-performance affinity chromatography. *Clinical Pediatric Endocrinology,* 18, 41-49.

Hayakawa, K., & Nagamine, T. (2009). Effect of fucoidan on the biotinidase kinetics in human hepatocellular carcinoma. *Anticancer Research,* 29, 1211-1218.

Hayakawa, K., & Nagamine, T. (2014). Fucoidan-dependent increased membrane component in HepG2: effect of fucoidan is not due to gene expression. *Cancer Genomics & Proteomics,* 11, 93-114.

Kögl, F., & Tönnis, B. (1936). Über das Bios-Problem. Darstellung von Krystallisierten Biotin aus Eigelb. *Hoppe-Seyler's Zeitschrift fur Physiologische Chemie*, 242, 43–73 (in German).

Kylin, H. (1915). Untersuchungen über die Biochemie der Meeresalgen. *Hoppe-Seyler's Zeitschrift fur Physiologische Chemie*, 94, 337-425 (in German).

Lynen, F. (1964). The pathway from “activated acetic acid” to the terpenes and fatty acids. *Nobel Lecture*, 11 December.

http://nobelprize.org/nobel_prizes/medicine/laureates/1964/lynen-lecture.pdf

Nagae, H. (1990) Effects of SSM, an extract from human type tubercle bacilli, on syngenic guinea pig tumors. *Nippon Ika Daigaku Zasshi,* 57, 235-243 (in Japanese).

Nagamine, T., Hayakawa, K., Nakazato, K., & Iha, M. (2015). Determination of the active transport of fucoidan derived from Okinawa Mozuku across the human intestinal Caco-2 cells as assessed by size-exclusion chromatography. *Journal of Chromatography B*, 997, 187-193. http://dx.doi.org/10.1016/j.jchromb.2015.05.026

Nilsson, L., & Kågedal, B. (1993). Co-purification of human serum lipoamidase and biotinidase: evidence that the two enzyme activities are due to the same enzyme protein. *Biochemical Journal*, 291, 545-551.

Oizumi, J., & Hayakawa, K. (1989). Liberation of lipoate by human serum lipoamidase from bovine heart pyruvate dehydrogenase. *Biochemical and Biophysical Research Communications*, 162, 658-663.

Schiffer, E., Housset, C., Cacheux, W., Wendum, D., Desbois-Mouthon, C., Rey, C., … Rosmorduc, O. (2005). Gefitinib, an EGFR inhibitor, prevents hepatocellular carcinoma development in the rat liver with cirrhosis. *Hepatology*, 41, 307-314.

Snedecor, G.W., & Cochran, W.G. (1967). *Statistical Methods. 6th ed.* Ames: The Iowa State University Press.

Snell, E.E., Strong, F.M., & Peterson, W.H. (1937). Growth factors for bacteria. VI: Fractionation and properties of an accessory factor for lactic acid bacteria. *Biochemical Journal*, 31, 1789–1799.

Stanley Tsai, C., Burgett, M.W., & Reed, L.J. (1973). α-Keto acid dehydrogenase complexes XX. A kinetic study of the pyruvate dehydrogenase complex from bovine kidney. *Journal of Biological Chemistry*, 284, 8348-8352.

Terentyeva, E.A., Hayakawa, K., Tanae, A., Katsumata, N., Tanaka, T., & Hibi, I. (1997). Urinary biotinidase and alanine excretion in patients with insulin-dependent diabetes mellitus. *European Journal of Clinical Chemistry and Clinical Biochemistry,* 35, 21-24.

Table 1. Summary of some differences in the effects on the cellular growth among fucoidans.*

Cells	Liver cell lines			Urinary bladder cells	
Fucoidans	Hc	HuH-6	HepG2	HuH-7	T24	5637
Ireland's-	X	X	X	X	–	–
Okinawa-	NE	◎	NE	NE	NE	NE
Okinawa-SMW-fucan	GA	GA	GA	GA	GA	GA
Noto-Ishi-Mozuku	NE	NE	◎	◎	—	–
Noto-Silky-Mozuku	NE	NE	◎	◎	—	–
Japan Sea Iwa-	NE	NE	○	○	—	–
Kombu	NE	NE	○	○	—	–

Mekabu	NE	NE	○	○	—	—

* Cells: Hc, undifferentiated fetal normal cells; HuH-6, undifferentiated-type pediatric cancer cells; HepG2 and HuH-7, well-differentiated-type adult cancer. Urinary bladder cancer cells; T24 (82 y, female; Sweden; Caucasian) and 5637 (68 y, male; Caucasian; Memorial Sloan Kettering Cancer、N.Y., USA) were both well-differentiated-type adult cancer. X: acute cell death; NE, not effective; GA, growth activation; ◎ and ○, well- and moderately-effective in the growth retardation.

Figure Caption

Figure 1. Prevention of the liver-tumor formation in SD rat by the feed containing Silky-Mozuku. Upper livers (C1, C2, C3); tumor-induced control without taking Mozuku. Lower livers (S1, S2, S3); sample with taking Mozuku in the feed for three weeks. Left-side photograph was taken from the abdominal side. Right-side photograph was also taken from the back side. Liver of C3 showed no apparent tumor on the surface, but unexpectedly contained a large tumor (1.5 cm) in the internal part of the liver after operation by scalpel (data not shown). The upper three cancer livers were larger in size (volume) than the lower prevented livers, however the wet weights were similar (data not shown). Therefore, cancer tissues might be smaller in the specific gravity than normal tissues. Other experimental conditions were as described in the Materials and Methods section.

IJRD International Journal of Research & Development Organisation

ISSN: 2455-7676

Journal of Biological Science