Infinimin® Immunity Multivitamin Executive Presentation

an Infinitum Health, LLC product

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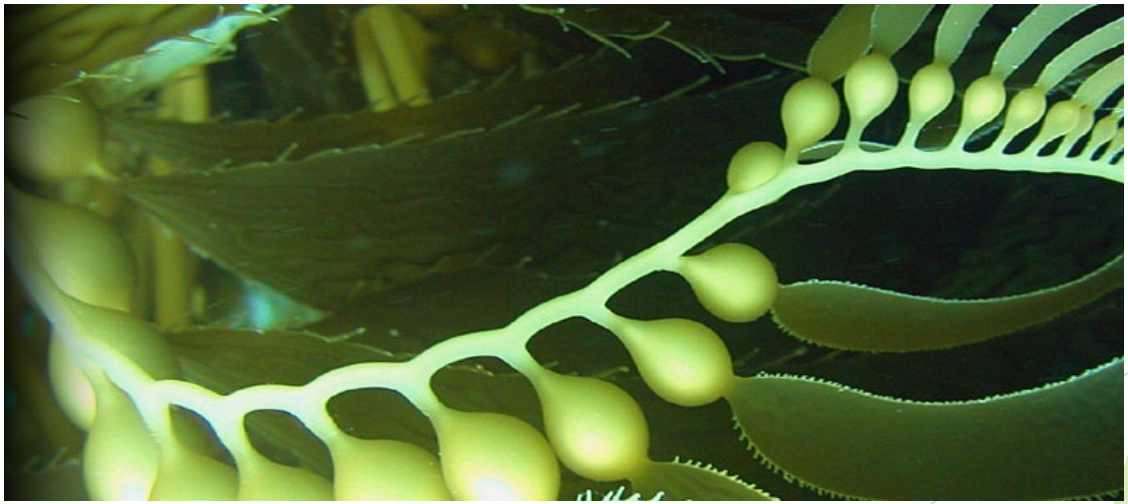
Owner/Founding Director

FDA Disclaimer

*These statements have not been evaluated by the Food and Drug Administration (FDA). These products are not intended to diagnose, treat, cure or prevent any disease. These powerpoint materials (and its reference links) are not to be used as a substitute for medical advice, diagnosis or treatment of any health condition or problem. Users of this powerpoint (and its reference links) should not rely on information provided for their own health problems. Any questions regarding your own health should be addressed to your physician or other healthcare provider. We encourage you to read through our research to aid in making an informed decision to buy.

Infinitum Health, LLC Value Proposition

"To provide natural integrative medicine products, with evidence based research, and aid in providing a healthful existence, forever, without limits."



Executive Summary - Company

Infinitum Health, LLC was founded in March, 2013. Infinitum Health, LLC was created to deliver high quality integrative medicine products to increase the overall health of the consumer.

As Infinitum Health, LLC grows, we plan to have strong evidence-based research behind every product to help support its use, further its research, and expand its reach across the globe. We feel that by providing the evidence-based research to the consumer, they can be empowered and inform themselves on our products and their related health benefits. We look forward to collaborating with our consumer in providing a "healthful existence, forever, without limits."

Current Funding Needs

- Infinitum Health, LLC has received dramatic customer testimonials on one of it's flagship products, Infinimin®.
- These testimonials include anticancer, antiviral, anti-diabetic, and energy promotion.
- **Formally moved from Supplement company to Biotechnology company with focused niche: Oncology**
- 2 Preclinical studies performed, 13 cancer cell lines showing anticancer properties
  - Natural Immune Systems (NIS), Inc
    - "A statistically significant reduction in the viability of all 4 cancer cell lines (A-172 - glioblastoma, DU-145 - prostate, A-549 - lung, and A-375 melanoma)
    - Gitte Jensen, PhD – Principal Investigator
  - Mayo Clinic
    - "A statistically significant reduction in nine of our most broad cancer cell types (LN18 – glioblastoma (MCF7 - breast, MC38 - colon carcinoma, B16 - melanoma, BM185 – leukemia, 4T1 - mammary tumor, CT26 - colon, 6C3HED – lymphosarcoma, SCC VII - squamous cell carcinoma)
    - David Lott, MD – Principal Investigator
- ***Executive Funding Request: $2,000,000***
- ***Executive Funding Strategy: Funding request to aid movement from Preclinical to Clinical Phase II & III testing***

Product Executive Summary - Infinimin®

- Infinimin®, is a premium health maintenance ultravitamin. It combines a full multivitamin panel with a patented blend of unique extracts from around the world creating a potent effective and efficient complement to an individual's diet.
- Inifinimin® has extracts that appear to promote dramatic immuno-stimulating and immune-modulating properties as well as a base essential multivitamin panel for general health maintenance needs
- The extracts in the patented blend have over 1400 research articles illustrating their promising health benefits.
- The extract patented blend has a combination of over 5000 years of history of use with Traditional Chinese Medicine, Brazilian Medicine, and Ayurvedic Medicine and 100 years of efficient Western medicine use, bringing you a truly holistic ultravitamin.

Infinimin®- Label

- Simple, elegant, and impactful labeling and formulation for “eye catching” consumers
- Key extracts and ingredients highlighted
- Good Manufacturing Practices (GMP) as certified quality designation
- Simple, memorable tagline, “… health, forever, without limits”

Market Space – Multivitamin/Herbal

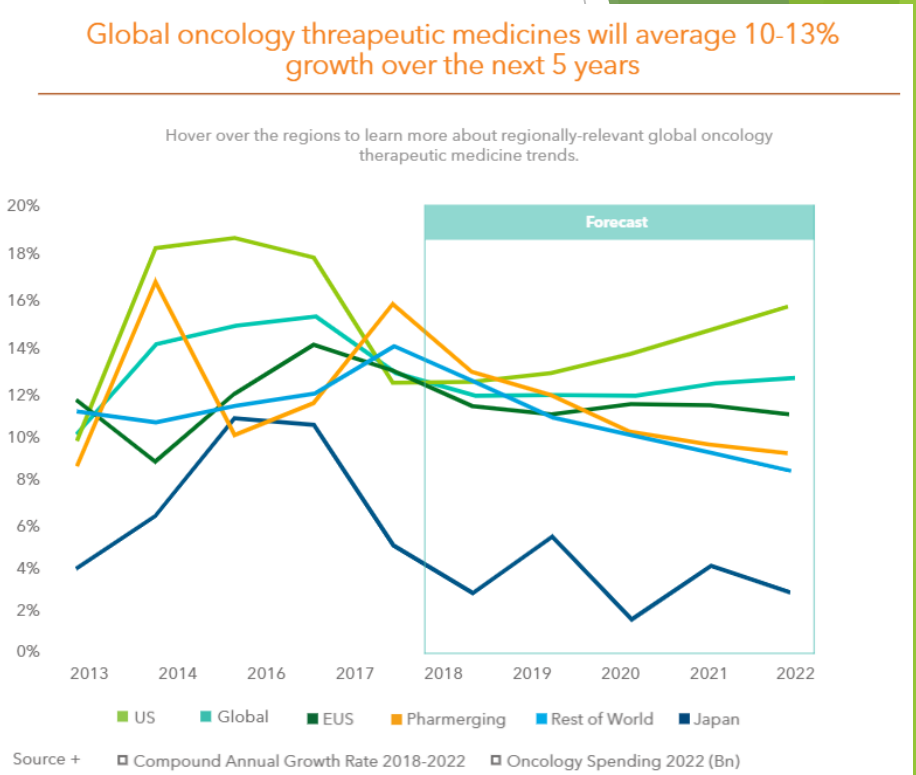
- Multivitamin, Herb/Botanical, & Natural and Organic Industry
  - $102 Billion market
- Key Leaders (Multivitamin)
  - Centrum® (Pfizer)
    - ~300 Million
  - One-A-Day® (Bayer)
    - ~400 Million
- No known immuno-supportive multivitamins on the market
  - Effectively managing a bridge between “Vitamins and Minerals” and “Herbs and Botanicals” market spaces

By Product Category	2007 ($Billion)	2008($Billion)	2008Growth
Vitamins & Minerals	29,810	31,980	7%
Herbs & Botanicals	20,260	20,900	3%
SHM&S Supplements	22,160	23,670	7%
Total Supplements	**72,230**	**76,550**	**6%**
Natural & Organic Food	63,230	70,800	12%
N&O Personal Care/HH	24,310	27,100	11%
Functional Food	90,110	95,350	6%
Total Nutrition Sales	**249,890**	**269,800**	**8%**

"Global Nutrition Sales Expand 8% in 2008 Despite Economic Storm." Nutrition Business Journal. Nov/Dec, 2009 Issue

Market Space - Oncology

- Multivitamin, Herb/Botanical, & Natural and Organic Industry
  - $133 Billion market (2017)
  - $200 Billion market (2022)
    - 13% YOY Growth, 5 years
- No known immuno-supportive multivitamins on the market
  - Anticancer properties
  - Chemotherapy complements
  - Safe, non-toxic
  - Cheap cost



Source: IQVIA Institute, Dec 2017Chart notes: Spending Growth in Constant US

Marketing

- Highest ROI is word of mouth*
- Customer Testimonials
  - Whitney McLennan, Polycystic Ovarian Disease, IBD, Kidney Tumor, Gall Bladder Stones, Infertile
  - Polycystic Ovarian Disease cleared up, Tumor eliminated, and pregnant (4 month timeframe of taking Infinimin®)
  - http://www.infinitumhealth.com/our-story
- Research
  - Infinitum Health Preclinical Proof of Concept Anti-Cancer Study
  - *"A statistically significant reduction in the viability of all 4 cancer cell lines (glioblastoma, prostate, lung, and melanoma) was seen following treatment with Infinimin®."*
- Professional Athletics
  - Ginger Huber, sponsored professional high diver, currently competing in Red Bull® High Diving competitions, High Diving currently under review by International Olympic Committee for 2020 Olympics
- Social Media
  - Facebook, Instagram, Twitter
  - 10,000 likes on Facebook, active community sharing stories on health promotion from Infinitum Health, LLC

*95% of sales were sourced by word of mouth, referrals, and recommendations

Intellectual Property

- 1 Patent Pending
- “Nutritional Supplement to Complement Cancer Therapy”
- https://www.google.com/patents/US20160038530

Nutritional Supplement to Complement Cancer Therapy

US 20160038530 A1

Publication number	US20160038530 A1
Publication type	Application
Application number	US 14/454,548
Publication date	Feb 11, 2016
Filing date	Aug 7, 2014
Priority date ⓘ	Aug 7, 2014
Inventors	Kevin Engholdt
Original Assignee	Kevin Engholdt
Export Citation	BiBTeX, EndNote, RefMan

Classifications (28)

External Links: USPTO, USPTO Assignment, Espacenet

ABSTRACT

A nutritional or dietary supplement composition that strengthens and promotes health through the prevention, stabilization, reversal and/or treatment of cancers, chemotherapy, and/or radiation therapy related complications. The nutritional or dietary supplement composition may likewise reduce the risk and/or prevalence of cancerous cells in addition to providing complement to cancer related therapies. The essential ingredients of the nutritional or dietary supplement composition are vitamin A, vitamin B6, vitamin B12, vitamin C, vitamin D3, vitamin E, calcium, thiamin, riboflavin, niacin, folic acid, pantothenic acid, fucoidan (extract from seaweed species such as, but not limited to; *laminaria japonica* and *undaria pinnatifida*), beta-glucans (extract from mushroom species such as, but not limited to; *ganoderma lucidum* and *grifola frondosa*), and antioxidants (from vegetable, fruit, or herb such as, but not limited to; acai berry (*Euterpe oleracea*), sumac, cloves, and sorghum). The essential ingredients are preferably provided in a capsule or tablet form suitable for oral ingestion. Preferably the composition is taken in the form of 3 tablets taken once daily.

- 2 Provisional Patents applied for

Infinimin® Price(s)

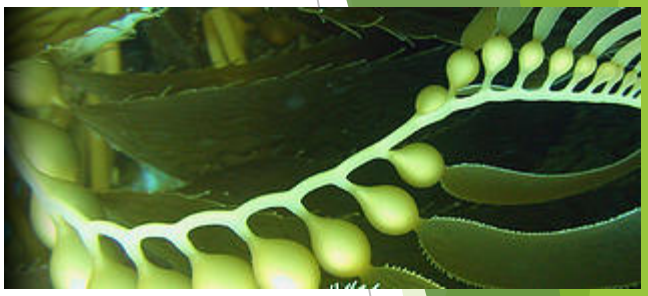
- Infinimin® Suggested Retail Sale Price
  - $44.99
- Infinimin® Wholesale Price
  - $19.99
- Margin: 56%
- Price is extremely competitive to Centrum® and One-A-Day® with fucoidan, beta glucans, and acai raw material, providing true health benefit (source: Nutrition Business Journal, Jan, 2011)
  - ~$110
- Price ability to increase 1000% due to laws for pricing botanical drugs
  - Evidence based trials required

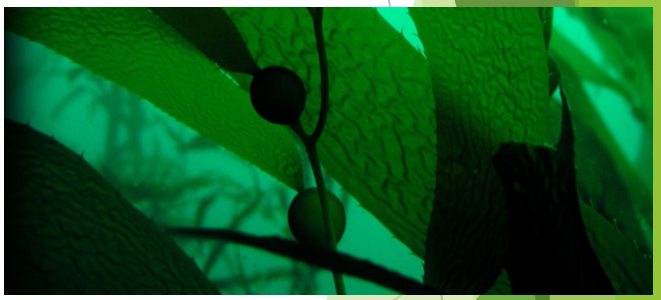
Audience / Distribution

- Informed, health conscious individuals
- Patients of recent significant diagnoses such as cancers, diabetes, obese, viral (HSV, HIV, flus, cold), as well as individuals recently genetically tested to have future diagnoses.
- Health/Nutrition stores
  - Hi-Health, GNC, VitaminShoppe, VitaminWorld
- Large Market Stores – Natural Focus – Future Market
  - Whole Foods, Sprouts, Fresh N Easy
- Online
  - Amazon.com, Drugstore.com, Infinimin.com
- Physician Led Practices:
  - Target patient populations: oncology, diabetes, cholesterol, obesity, fatigue, fertility, virility

Patented Blend - Fucoidan

- **Fucoidan - from seaweed - becoming the most potent immunity stimulator on earth**
- In our product, this extract from brown seaweeds [from Kombu (昆布, ***laminaria japonica***), Wakame (ワカメ, ***undaria pinnatifda***) and Bladderwrack (***fucus vesiculosous***)] which have shown significant promising immunostimulant properties, including anticancer, antiviral, and anti-inflammatory properties[1]*.
- Fucoidan has blossomed the past 10 years in terms of evidence based research and is looking to be the most promising health support extract in the world from our ancient oceans.
- Having evolved and survived an intense environment of our oceans for over 3 billion years, seaweeds are one of our oldest and most beneficial plants to complement our overall daily diet.[2]





[1]Bo Li, Fei Lu, Xinjun Wei and Ruixiang Zhao; Fucoidan: Structure and Bioactivity. Molecules 2008, 13, 1671-1695

[2]Marcel Tutor Ale, Jørn D. Mikkelsen and Anne S. Important Determinants for Fucoidan Bioactivity: A Critical Review of Structure-Function Relations and Extraction Methods for Fucose-Containing Sulfated Polysaccharides from Brown Seaweeds. Mar. Drugs 2011, 9, 2106-2130

Patented Blend – ß-glucans

- **ß-glucans - from mushrooms - broad spectrum anti-aging and immune enhancers**
- Extracts (ß-glucans) from specific mushrooms have been shown to have multiple immune supporting properties, including anticancer, antidiabetic, and anti-inflammatory properties, [3]*.
- Maitake mushroom (舞茸, ***grifola frondosa***), Caterpillar mushroom (***cordyceps sinensis***) and Reishi mushroom (靈芝, ***ganoderma lucidum***), also known as "Immortal Mushroom", have been used in Asian cultures for thousands of years for what is now known as one of the broadest immune-modulators in history[3].
- Mushrooms are one of the oldest land based plants and are the "decomposers" of ecosystems recycling nutrients and allowing new growth to occur. It is not surprising that they seem to balance ecosystems and appear to balance physiological systems as well.

[3] Wasser, S. Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. Applied Microbiology and Biotechnology. March 2011, Volume 89, Issue 5, pp 1323-1332

Wasser, S. International Journal of Medicinal Mushrooms. Vol 12 Issue 3 2010.

Patented Blend – Acai Berry

- **Acai Berry – one of the most potent antioxidants in the world**
- The Acai berry (***euterpe oleracea***) from Brazil has shown to be the most potent anti-oxidant (anti-aging) in the world.
- In the lab, Acai has been found to possess remarkable antioxidant activity in human cells, even when diluted to one part per trillion†.
- Acai, among all antioxidant extracts, has the most significant impact and supports your cells to stay alive. This support is shown by what is know as the oxygen radical absorbance capacity, or ORAC. While other analytic methodologies may be used, ORAC is often considered preferable because of its biological relevance to antioxidant action in vivo (in living organisms). It measures both the degree and speed with which a certain food inhibits the action of an oxidizing agent, then integrates these two measurements into a single value, producing an accurate assessment of different types of antioxidants of different strengths.

ORAC: TOP 5 - RANKED ANTIOXIDANT FRUITS (ORAC units per 100 grams (about 3.5 oz))*

Açaí berries	18,400
Pomegranates	10,500
Blackberries	5,100
Bilberry	4,200
Blueberries	3,200

†Schauss AG, Wu X, Prior RL, Ou B, Huang D, Owens J, Agarwal A, Jensen GS, Har, AN, Shanbrom E. Antioxidant capacity and other bioactivities of the freeze-dried Amazonian palm berry, Euterpe oleraceae mart. (acai). Agric Food Chem. 2006 Nov 1;54(22):8604-10

Schauss AG, Wu X, Prior RL, et al. Phytochemical and nutrient composition of the freeze-dried Amazonian palm berry, Euterpe oleracea Mart. (Açai). J Agric Food Chem. 2006 Nov 1;54(22):8598-603.

Enten, Roni. Life Extension. June, 2010

*There are other higher antioxidant foods such as cloves and sumac, however, from a business standpoint, the smell of cloves and/or sumac does not appeal to the general population for a multivitamin.

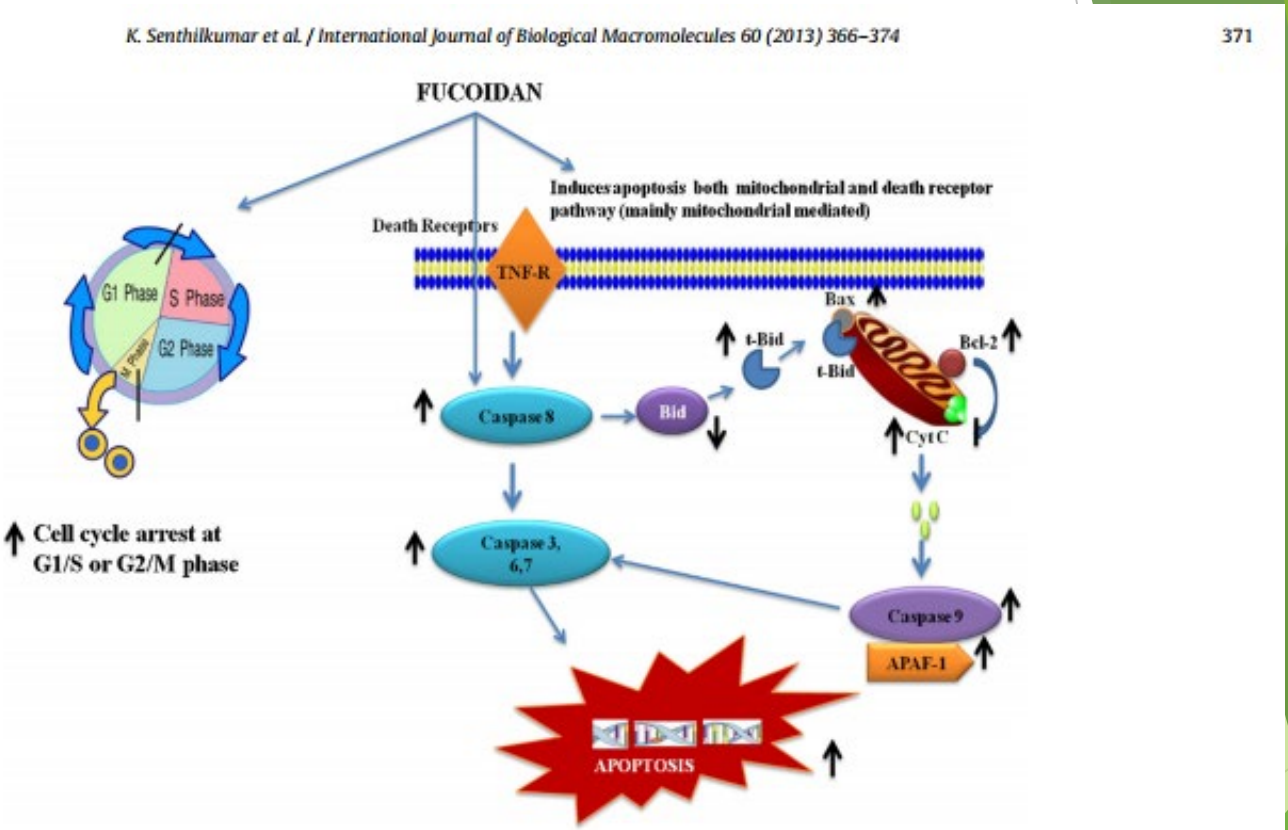
Multivitamin Panel

- Vitamin complement increases activity, specifically, tumor inhibition and suppression (Vitamin C & Fucoidan; Vitamin C and Maitake) based in in-vitro results†,‡
- Vitamin increases the absorption of larger polysaccharides, specifically, ß-glucans from mushrooms (e.g. Reishi and Maitake)‡
- Multivitamin is a known market space and accepted supplement delivery model
  - **Vitamin A** helps form and maintains healthy teeth, bones, soft tissue, mucous membranes, and skin.
  - **Vitamin B6** helps break down proteins, helps maintain normal nerve function and form red blood cells.
  - **Vitamin B12** is essential for metabolism. It also helps form red blood cells and maintains the central nervous system.
  - **Vitamin C** promotes the health of teeth and gums as well as wound healing.
  - **Vitamin D** helps the body absorb calcium, which is important for healthy teeth and bones. It also helps maintain proper blood levels of calcium and phosphorus.
  - **Vitamin E** is an antioxidant that plays a role in the formation of red blood cells.
  - **Vitamin K** is required for normal coagulation of the blood. Some studies suggest that it helps maintain bone health in the elderly.
  - **Riboflavin (B2)** works with the other B vitamins. It is important for body growth and the production of red blood cells.
  - **Thiamine (B1)** helps the body's cells change carbohydrates into energy. It is also essential for heart function and healthy nerve cells.

† Yasukazu Saitoh, Yuko Nagai, Nobuhiko Miwa. Fucoidan-Vitamin C complex suppresses tumor invasion through the basement membrane, with scarce injuries to normal or tumor cells, via decreases in oxidative stress and matrix metalloproteinases. International Journal of Oncology. Nov 2009 Vol 35 No 5.

‡ Fei Zhao, et. Al. Synergistic Apoptotic Effect of D-Fraction From Grifola frondosa and Vitamin C on Hepatocellular Carcinoma SMMC-7721 Cells. Integrative Cancer Therapies. 2017. Vol. 16(2) 205-214.

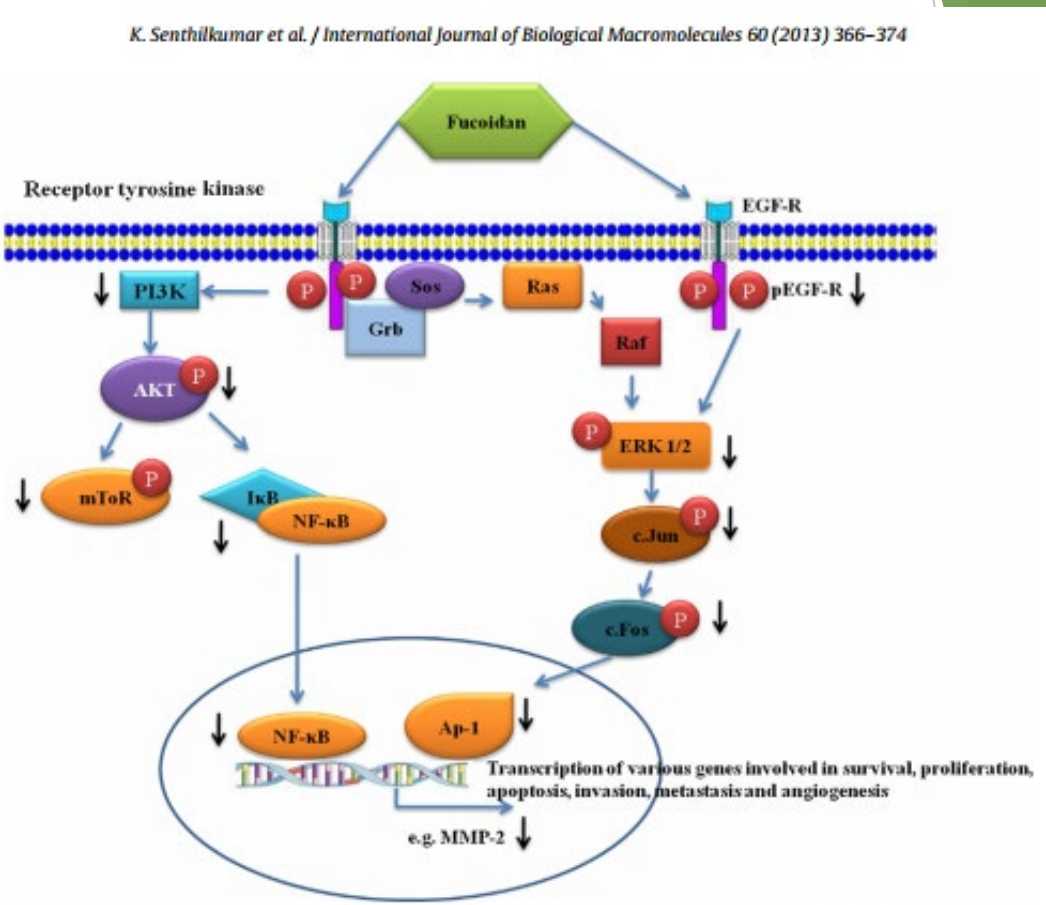
Proposed Mechanism of Action – Fucoidan[1]



- *"**Fig. 2**. Schematic representation of cell cycle arrest and apoptosis mechanisms of fucoidan. Fucoidan arrest cell cycle at G1/S or G2/M phase. Apoptosis occurs via extrinsic and extrinsic pathways. Fucoidan can activate both the pathway mediated apoptosis. Black color small arrow indicates downregulation and upper arrow indicates upregulation of protein activation by fucoidan."*

1. Brown seaweed fucoidan: Biological activity and apoptosis, growth signaling mechanism in cancer. K. Senthilkumar et al. / International Journal of Biological Macromolecules 60 (2013) 366–374

Proposed Mechanism of Action – Fucoidan[1]



- *"**Fig. 3**. Schematic representation of fucoidan on some growth signaling molecules. PI3K/AKT mediated pathway and EGR-R pathways plays major role in cancer. Fucoidan regulates growth signaling molecules involved in the cell survival, proliferation, apoptosis, invasion, metastasis and angiognenesis. Downward black color arrow indicates that decreased activity of the molecules by fucoidan."*

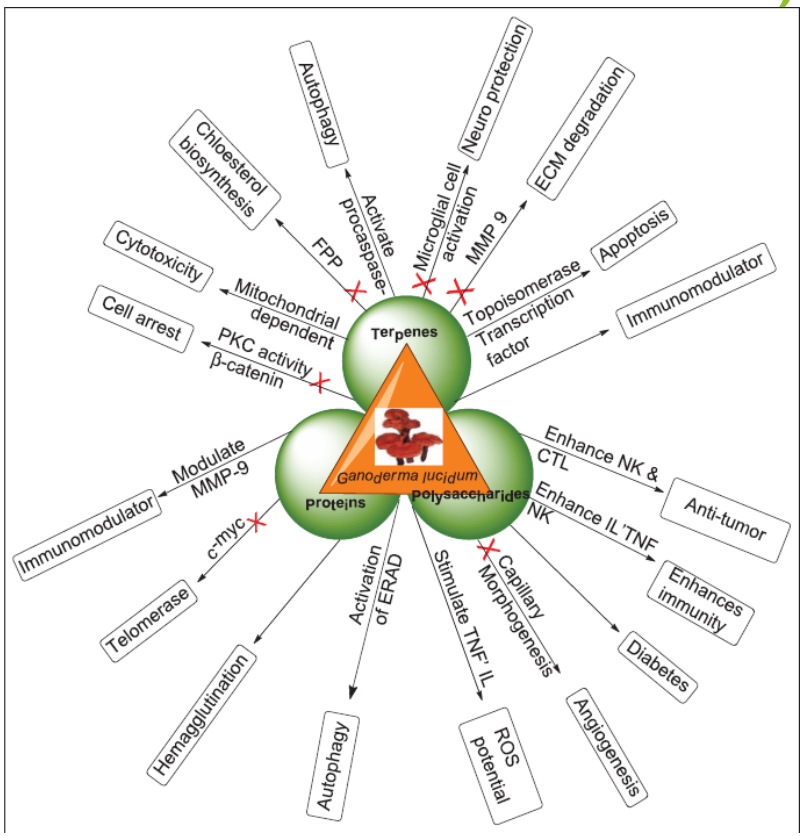
1. Brown seaweed fucoidan: Biological activity and apoptosis, growth signaling mechanism in cancer. K. Senthilkumar et al. / International Journal of Biological Macromolecules 60 (2013) 366–374

Proposed Mechanism of Action – ß-glucans (*ganoderma lucidum*)[1]

- The major bioconstituents of *G. lucidum* are mainly terpenes, polysaccharides, and ß-glucans.
  - Anticancer, antiviral, and antidiabetic effects. Anticancer is the focus of this proposal
- The bioconstituents of *G. lucidum* activate plasma membrane receptors and initiate various downstream signaling leading to nuclear factor-κB, phosphoinositide 3-kinase, Akt, and mammalian target of rapamycin in cancer.
- The bioconstituents regulate the expression of various genes involved in cell cycle, immune response, apoptosis, and autophagy in lung cancer.

1. Ganoderma lucidum targeting lung cancer signaling: A review
Balraj Singh Gill[1], et. Al, Department of Biotechnology, Doaba College, Jalandhar, India[3]Centre for Plant Sciences, Central University of Punjab, Bathinda, India *Tumor Biology*, Vol 39, Issue 6, First published date: June-27-2017

Proposed Mechanism of Action – β-glucans (*ganoderma lucidum*)[1]



- **"Figure 1.** Action of mechanism of myco-constituents of *Ganoderma lucidum* in signaling in cancer. Major constituents of *G. lucidum* are terpenes, polysaccharides, and proteins involved in cancer signaling. Terpenes arrest cell cycle and apoptosis and enhance the immune system and exhibit anti-cancerous property with involving different adapter molecules. Multifunctional nature of various terpenes stimulates the immune system and target apoptosis through activating caspases (mitochondrial-dependent). Protein and polysaccharides stimulate the expression of various factor in immune system and scavenging free radicals vital in cancer signaling and diabetes."

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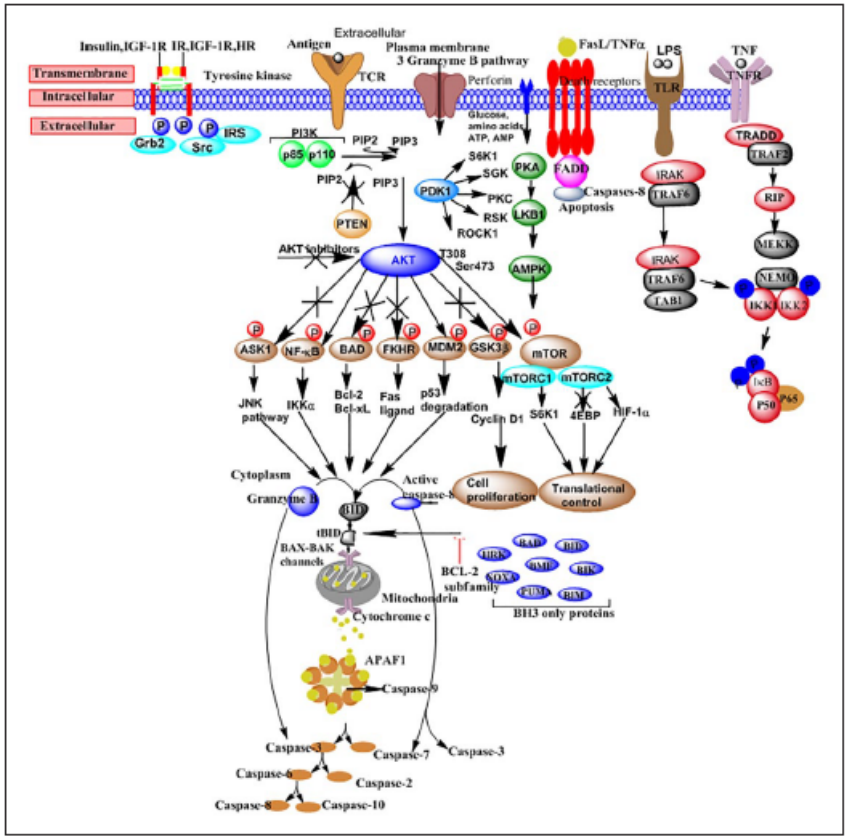
Proposed Mechanism of Action – β-glucans (*ganoderma lucidum*)[1]



- ***"Figure 3**. Signaling cascade initiated by Ganoderma lucidum and its bioconstituents in cancer. Upon receiving stimulus such as growth factor initiates the activation of different receptors in the plasma membrane particularly RTKs. RTK phosphorylation leads to dimerization and activation of adapter molecules, initiating the process of apoptosis in cancer. Upon stimulus, RTKs activates PI3K/Akt/mTOR pathway with other proteins modulating the behaviour of action in cancer signaling. G. lucidum modulates and control the expression of various signaling factors in both intrinsic and extrinsic apoptosis, depending upon a signal received."*

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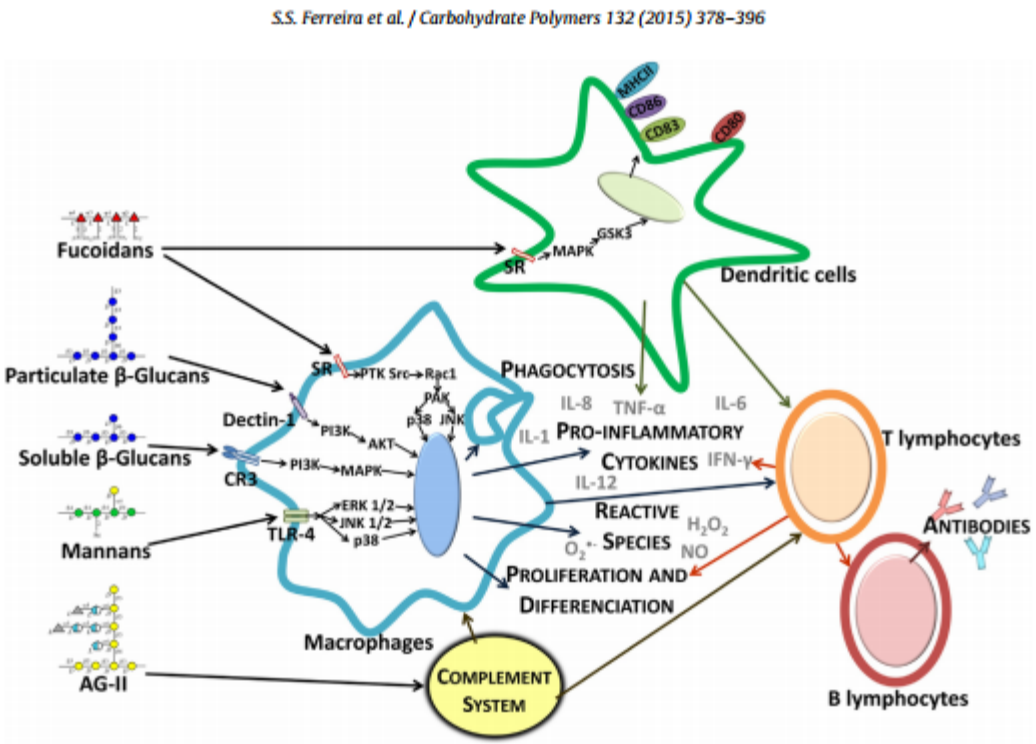
Immunostimulatory Activation by Polysaccharides Proposal[1]



*"**Fig. 1**. Illustration of immune system activation by immunostimulatory polysaccharides after interaction and trigger of several molecular/cellular events. Abbreviations: AG-II, type II arabinogalactans; Akt, protein kinase B; CD, cluster of differentiation; CR3, complement receptor 3; ERK 1/2, extracellular signal regulated kinase 1/2; GSK3, glycogen synthase kinase 3-; H2O2, hydrogen peroxide; IFN, interferon; IL, interleukin; JNK 1/2, Jun N-terminal kinase 1/2; JNK, c-Jun N-terminal kinase; MAPK, p38 mitogenactivated protein kinase; MHCII, major histocompatibility complex class II; NO, nitric oxide; O2•−, superoxide anion; PAK, p21-activated kinase; PI3K, phosphatidylinositol-3 kinase; PTK, protein-tyrosine kinase; Rac 1, Ras-related C3 botulinum toxin substrate 1; SR, scavenger receptor; Src, proto-oncogene protein kinase; TLR-4, toll-like receptor 4; and TNF-, tumour necrosis factor ."*

1. S.S. Ferreira et al. / Carbohydrate Polymers 132 (2015) 378–396

Effects of fucoidans on cancer cell survival and proliferation mechanisms[1]

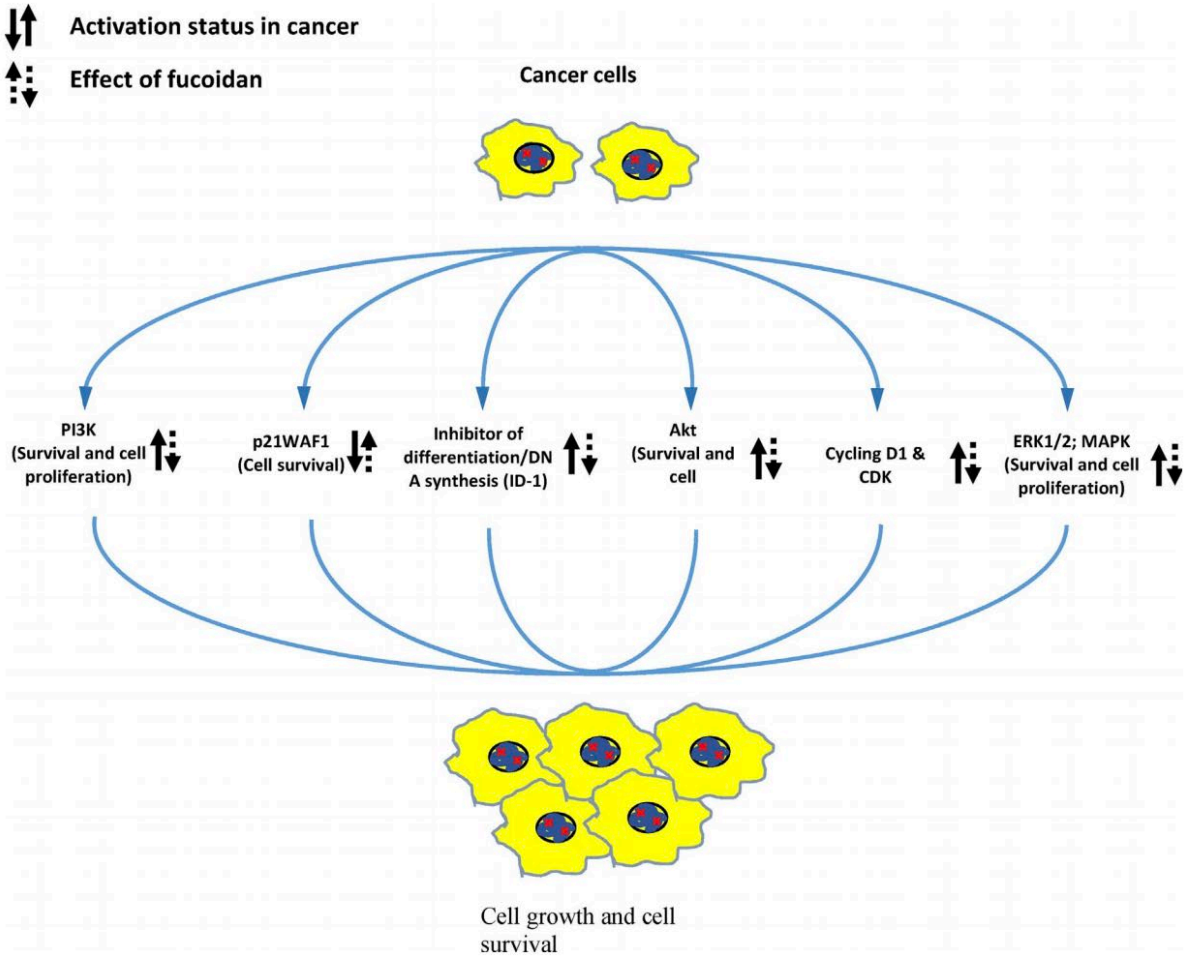
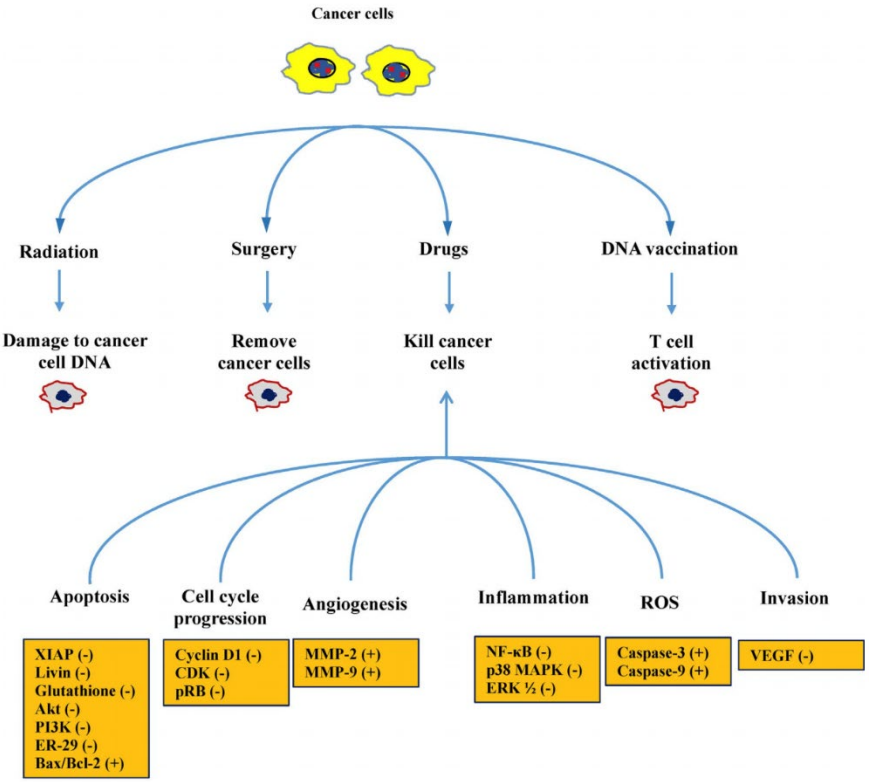


Fig. 2. Effects of fucoidans on cancer cell survival- and proliferation-related signal transduction pathways.

- *Numerous pathways impacted in reducing the cancer cell growth and inducing apoptosis*

1. K.K.A. Sanjeewa et al. Carbohydrate Polymers 177 (2017) 451–459

Brown Seaweed Polysaccharides ant-cancer cell mechanisms Proposal[1]



(-) = Polysaccharides down-regulate the expression of signaling molecules or proteins.
(+) = Polysaccharides up-regulate the expression of signaling molecules or proteins.

Fig. 3. Common cancer-preventive methods and key anti-cancer cell mechanisms reported for brown-seaweed polysaccharides.

*"**Fig. 3**. Common cancer preventative methods and key anti cancer cell mechanisms reported for brown seaweed polysacchardies*

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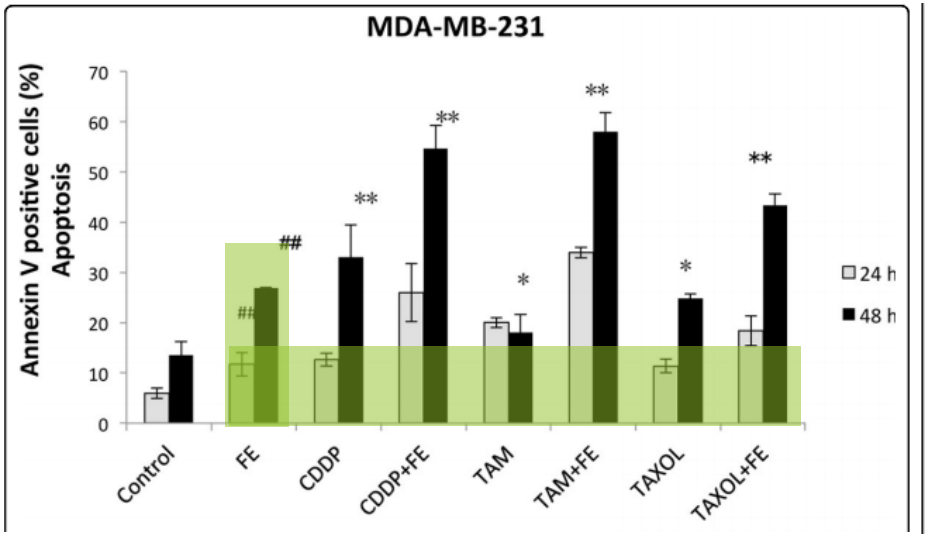
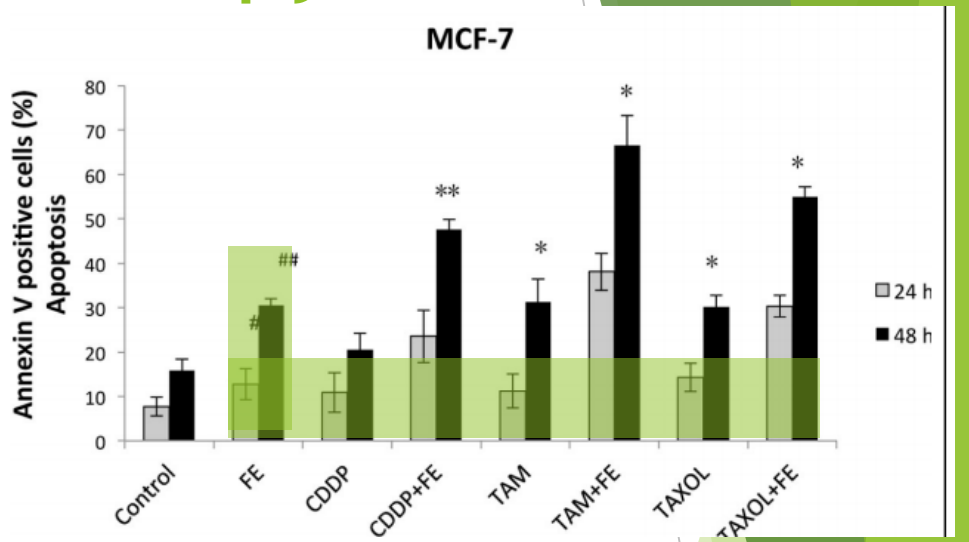
Key Research Highlighted – Fucoidan and Chemotherapy[1]

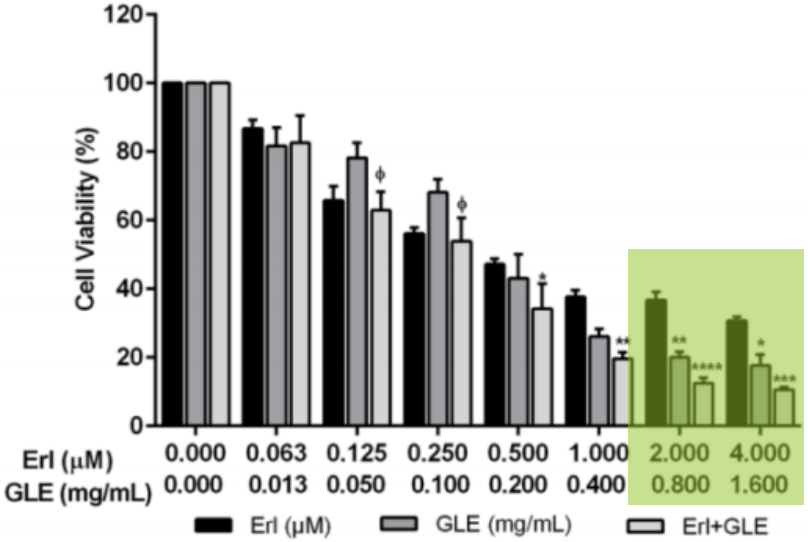




- Fucoidan (FE), Cisplatin (CDDP), Tamoxifen (TAM), Paclitaxel (TAXOL) were used on two breast cancer cell lines; MDA-MB-231 and MCF-7
- "Synergistic" apoptosis of cancer cells using fucoidan + (Cancer Therapy)
- **Amazingly – FE alone was just as successful as CDDP, TAM, and TAXOL alone.**
  - Average cost to bring a new drug to market is $802 million[2] . CDDP, TAM, and TAXOL provided ~$2.4 billion dollars of economy to the United States.
  - Author pressure to publish, authors make no mention of this, only of the "synergism" of combinations
- *"Figure 1. Synergistic induction of apoptosis by co-treatment and analysis of apoptotic cells by annexin/PI double-staining using theIN Cell Analyzer 1000. MDA-MB-231 and MCF-7 cells were treatedfor different times with 200 µg/mL FE alone or 200 µg/mL FEin combination with 5 µM CDDP, 10 µM TAM or 2.5 nM TAXOL after 48 h of treatment. All results were obtained from three independent experiments. A significant difference from control is indicated by $p < 0.05$ (#) or $p < 0.01$ (##); a significant difference from single treatments is indicated by $p < 0.05$ (*) or $p < 0.01$ (**)."*

1. Shirahata et al. BMC Proceedings 2013, 7(Suppl 6):P70
2. DiMasi, et. Al. The price of innovation: new estimates of drug development costs. Journal of Health Economics 22 (2003) 151–185

Key Research Highlighted – *ganoderma lucidum* and Chemotherapy[1]



- Erlotinib (Erl) and *ganoderm lucidum (GLE)* on breast cancer cells SUM-149 and SUM-102
- Findings "strongly suggest" that ganoderma be comgined with other agents against breast cancer.
- **However, GLE, amazingly, by itself has dramatic anticancer properties, with no side effect.**
  - Authors don't highlight, potentially due to publishing pressure
- *"Figure 1. Effect of Erl/GLE in EGFR-overexpressing cells. A, B. SUM-102 and SUM-149 cells were treated with GLE for 72h. Cell viability was calculated as in materials and methods. IC50 was obtained from dose response curve fittings using non-linear regression. C. SUM-149 cells were treated with GLE for 72h, to measure BrdU incorporation. Significance against vehicle (*) (P≤0.05). SUM-149 cells were treated with Erlotinib, GLE or Erl/GLE for 72h. D, E. Cell viability and CIs were calculated. Significance against Erlotinib (*) or GLE (ϕ) (P≤0.05). Columns represent means ± SEM. CIs were calculated based on the IC50 at a constant Erl:GLE ratio (1:1000) and at NCR (Erlotinib dilutions+0.05 mg/mL-GLE). CIs and fraction affected (Fa) calculated from the Erlotinib (E) and GLE (G) combinations were obtained using CompuSyn®. Fa-CI plot shows the interaction between drugs in function of Fa (original software output).*

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Key Research Highlighted – *Asian populations and outcomes*

- Japan* 98% of consumption of seaweed, 78% consumption of mushrooms
  - Lowest HIV rates on the planet
  - Lowest virus rates across broad spectrum of colds, flus, and herpes (HSV)
    - Lowest number of reported colds and flus of all countries worldwide
    - SARS virus was massive shock for culture that never gets virus sicknesses
  - Of the top 50 countries with best reported data for Cancer outcomes, Japan, holistically, has the 3rd lowest incidence
  - Other lifestyle factors also help
    - Minimal sugar intake in population
    - Minimal preservatives in food population
    - Culture of health and wellness
  - Culture of "moderation"

*Japan is one of the most studied Asian countries for public health due to access and credibility of its data(3).

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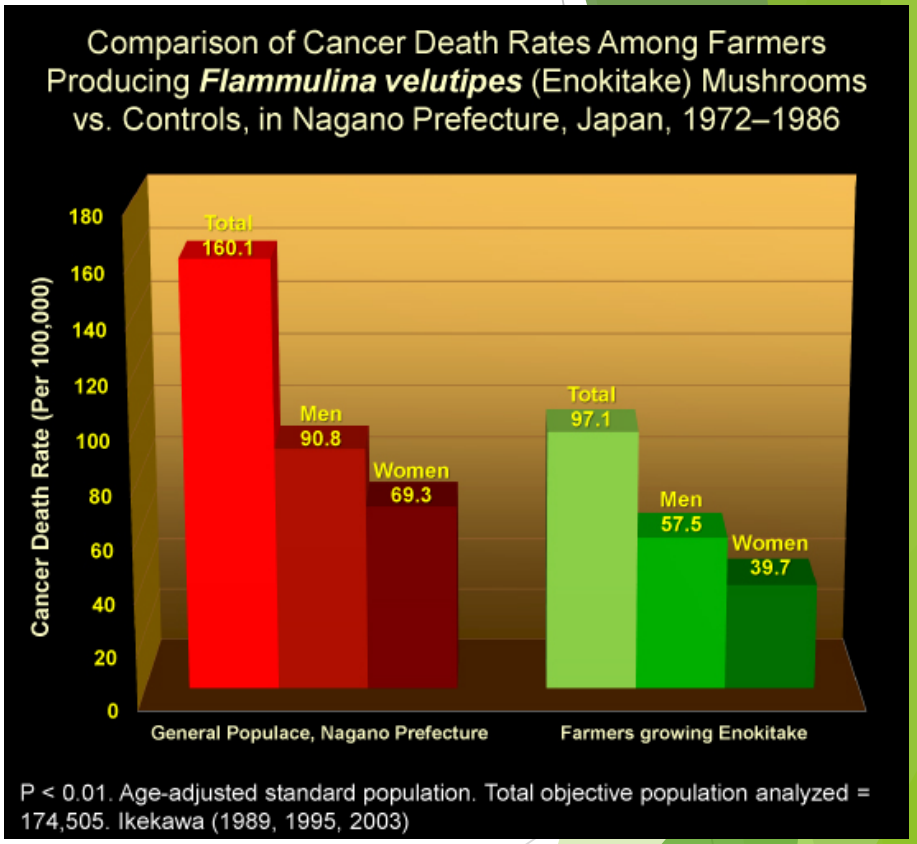
Key Research Highlighted – *Asian populations and outcomes*

- Japanese enoki mushroom (*Flammulina velutipes)* public health study (1969), triggered international review of mushrooms and capability for overall health, specifically cancers.
- Dr. Tetsuro Ikekawa, a former epidemiologist at the Research Institute of the National Cancer Center in Tokyo, wondered why the cancer rates in the Nagano Prefecture of Japan were abnormally low from 1972-1986, compared to surrounding provinces. Ikekawa found it was the center of enoki mushroom cultivation. A cluster within the population of Nagano died less frequently from cancer: enoki mushroom growers and their families. Since many enoki farmers gave their employees the bruised or blemished mushrooms that were deemed unattractive to shoppers, these Nagano citizens ate far more enoki mushrooms than their neighbors. Dr. Ikekawa surmised that their higher rate of enoki mushroom consumption correlated with the lower cancer death rate in Nagano Prefecture.
- At the time of the research, the average cancer death rate in the Nagano prefecture was 160 per 100,000. This rate dropped to 97 per 100,000, comparatively, in families of enoki growers (Ikekawa, et al,,1989, 2003). Men's cancer deaths decreased by 36.6 percent, and women in this cluster benefited from a 42.7 percent decrease in mortality from cancer. The population base in this study was around 175,000 people and was age-adjusted. By contrast, the United States currently records 173 deaths from cancer per 100,000 as of 2009 (The Henry J. Kaiser Foundation).
- While there are no clusters of enoki growers and enoki eaters to study in the U.S. like there are in Nagano, this Japanese study did inspire epidemiologists to study the effect of higher mushroom consumption as well as what extracts are causing such a response.
- Such research could support the widespread theory held by many mycologists and physicians that increased mushroom consumption can lower cancer fatality rates.



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Proposed Mechanism of Action Summary and Next Steps

- Two Examples of proposed mechanisms given
  - Fucoidan from seaweed
  - Beta Glucans and related mushroom compounds (*ganoderma lucidum*)
- Much of this proposed mechanism requires further research, but initial results appear very promising
- A. Fleming discovered penicillin (ironically from mushrooms) by accident and recognized the outcome for treatment of infections in 1928.
  - Use began immediately in 1929 due to U.S. Great Depression and European economy collapse, causing large scale disease outbreaks
- 40 years later, 1968, a formal mechanism of action was proven [1]
  - Penicillin breaks the 5-Glycine bridge of cell wall synthesis of bacteria
- Infinimin® is a combined essential multivitamin panel and patented blend of three seaweed species, three mushroom species, and a potent antioxidant berry, Acai.
  - The true mechanism of action, may not be known for a very long time for any of its proposed immune enhancing properties for anticancer, antiviral, antidiabetic, anti-aging, etc.
  - **What is known, however, is that it is safe and appears to work, on differing levels for different people.**

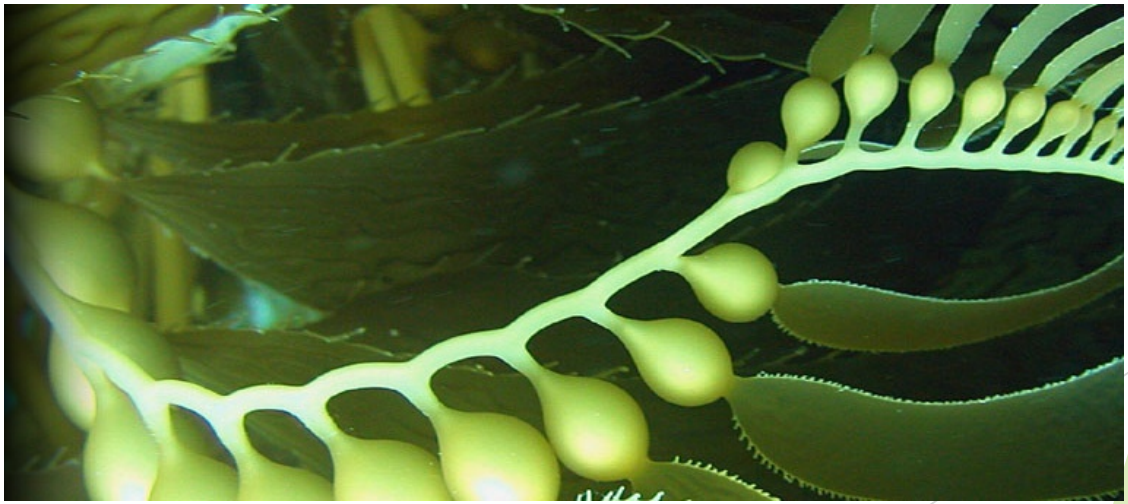
1. Mechanism of Action of Penicillin. R. Rogers, et. Al. The Journal of Biological Chemistry. Vol. 255, No. 9, Issue of May 10, pp. 3977-3986, 1980

Additional Products

- Infiniderm®
  - Natural daily lotion with a proprietary blend of anti-aging and dermal support for supple, healthy, young looking skin.
- Releaf®
  - Unique natural pain reliever proprietary blend supporting muscle aches, headaches, and chronic pain.
- Ancient Cleanse®
  - Natural face and body soap comprised of a proprietary blend for cleaning and exfoliating skin for a rejuvenating and healthful look.

Infinitum Health, LLC Value Proposition

"To provide integrative medicine products, with evidence based research, and aid in providing a healthful existence, forever, without limits."



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References/Backup

Fucoidan: Structure and bioactivity

Fucoidan refers to a type of polysaccharide which contains substantial percentages of L-fucose and sulfate ester groups, mainly derived from brown seaweed. For the past decade fucoidan has been extensively studied due to its numerous interesting biological activities. Recently the search for new drugs has raised interest in fucoidans. In the past few years, several fucoidans' structures have been solved, and many aspects of their biological activity have been elucidated. This review summarizes the research progress on the structure and bioactivity of fucoidan and the relationships between structure and bioactivity.

Important Determinants for Fucoidan Bioactivity: A Critical Review of Structure-Function Relations and Extraction Methods for Fucose-Containing Sulfated Polysaccharides from Brown Seaweeds

Seaweeds—or marine macroalgae—notably brown seaweeds in the class Phaeophyceae, contain fucoidan. Fucoidan designates a group of certain fucose-containing sulfated polysaccharides (FCSPs) that have a backbone built of (1→3)-linked α-l-fucopyranosyl or of alternating (1→3)- and (1→4)-linked α-l-fucopyranosyl residues, but also include sulfated galactofucans with backbones built of (1→6)-ß-d-galacto- and/or (1→2)-ß-d-mannopyranosyl units with fucose or fuco-oligosaccharide branching, and/or glucuronic acid, xylose or glucose substitutions. These FCSPs offer several potentially beneficial bioactive functions for humans. The bioactive properties may vary depending on the source of seaweed, the compositional and structural traits, the content (charge density), distribution, and bonding of the sulfate substitutions, and the purity of the FCSP product. The preservation of the structural integrity of the FCSP molecules essentially depends on the extraction methodology which has a crucial, but partly overlooked, significance for obtaining the relevant structural features required for specific biological activities and for elucidating structure-function relations. The aim of this review is to provide information on the most recent developments in the chemistry of fucoidan/FCSPs emphasizing the significance of different extraction techniques for the structural composition and biological activity with particular focus on sulfate groups

Current findings, future trends, and unsolved problems in studies of medicinal mushrooms

The target of the present review is to draw attention to many critically important unsolved problems in the future development of medicinal mushroom science in the twenty-first century. Special attention is paid to mushroom polysaccharides. Many, if not all, higher Basidiomycetes mushrooms contain biologically active polysaccharides in fruit bodies, cultured mycelium, and cultured broth. The data on mushroom polysaccharides are summarized for approximately 700 species of higher Hetero- and Homobasidiomycetes. The chemical structure of polysaccharides and its connection to antitumor activity, including possible ways of chemical modification, experimental testing and clinical use of antitumor or immunostimulating polysaccharides, and possible mechanisms of their biological action, are discussed. Numerous bioactive polysaccharides or polysaccharide–protein complexes from medicinal mushrooms are described that appear to enhance innate and cell-mediated immune responses and exhibit antitumor activities in animals and humans. Stimulation of host immune defense systems by bioactive polymers from medicinal mushrooms has significant effects on the maturation, differentiation, and proliferation of many kinds of immune cells in the host. Many of these mushroom polymers were reported previously to have immunotherapeutic properties by facilitating growth inhibition and destruction of tumor cells. While the mechanism of their antitumor actions is still not completely understood, stimulation and modulation of key host immune responses by these mushroom polymers appears central. Particularly and most importantly for modern medicine are polysaccharides with antitumor and immunostimulating properties. Several of the mushroom polysaccharide compounds have proceeded through phases I, II, and III clinical trials and are used extensively and successfully in Asia to treat various cancers and other diseases. A total of 126 medicinal functions are thought to be produced by medicinal mushrooms and fungi including antitumor, immunomodulating, antioxidant, radical scavenging, cardiovascular, antihypercholesterolemia, antiviral, antibacterial, antiparasitic, antifungal, detoxification, hepatoprotective, and antidiabetic effects.

References

Antioxidant capacity and other bioactivities of the freeze-dried Amazonian palm berry, Euterpe oleraceae mart. (acai).

The fruit of Euterpe oleraceae, commonly known as acai, has been demonstrated to exhibit significantly high antioxidant capacity in vitro, especially for superoxide and peroxyl scavenging, and, therefore, may have possible health benefits. In this study, the antioxidant capacities of freeze-dried acai fruit pulp/skin powder (OptiAcai) were evaluated by different assays with various free radical sources. It was found to have exceptional activity against superoxide in the superoxide scavenging (SOD) assay, the highest of any food reported to date against the peroxyl radical as measured by the oxygen radical absorbance capacity assay with fluorescein as the fluorescent probe (ORACFL), and mild activity against both the peroxynitrite and hydroxyl radical by the peroxynitrite averting capacity (NORAC) and hydroxyl radical averting capacity (HORAC) assays, respectively. The SOD of acai was 1614 units/g, an extremely high scavenging capacity for O2*-, by far the highest of any fruit or vegetable tested to date. Total phenolics were also tested as comparison. In the total antioxidant (TAO) assay, antioxidants in acai were differentiated into "slow-acting" and "fast-acting" components. An assay measuring inhibition of reactive oxygen species (ROS) formation in freshly purified human neutrophils showed that antioxidants in acai are able to enter human cells in a fully functional form and to perform an oxygen quenching function at very low doses. Furthermore, other bioactivities related to anti-inflammation and immune functions were also investigated. Acai was found to be a potential cyclooxygenase (COX)-1 and COX-2 inhibitor. It also showed a weak effect on lipopolysaccharide (LPS)-induced nitric oxide but no effect on either lymphocyte proliferation and phagocytic capacity.

Phytochemical and nutrient composition of the freeze-dried amazonian palm berry, Euterpe oleraceae mart. (acai).

Euterpe oleraceae is a large palm tree indigenous to the Amazon River and its tributaries and estuaries in South America. Its fruit, known as acai, is of great economic value to native people. In this study, a standardized freeze-dried acai fruit pulp/skin powder was used for all analyses and tests. Among many findings, anthocyanins (ACNs), proanthocyanidins (PACs), and other flavonoids were found to be the major phytochemicals. Two ACNs, cyandin 3-glucoside and cyanidin 3-rutinoside were found to be predominant ACNs; three others were also found as minor ACNs. The total content of ACNs was measured as 3.1919 mg/g dry weight (DW). Polymers were found to be the major PACs. The concentration of total PACs was calculated as 12.89 mg/g DW. Other flavonoids, namely, homoorientin, orientin, isovitexin, scoparin, and taxifolin deoxyhexose, along with several unknown flavonoids, were also detected. Resveratrol was found but at a very low concentration. In addition, components inluding fatty acids, amino acids, sterols, minerals, and other nutrients were analyzed and quantified. Total polyunsaturated fatty acid, total monounsaturated fatty acid, and total saturated fatty acids contributed to 11.1%, 60.2%, and 28.7% of total fatty acid. Oleic acid (53.9%) and palmitic acid (26.7%) were found to be the two dominant fatty acids. Nineteen amino acids were found; the total amino acid content was determined to be 7.59% of total weight. The total sterols accounted for 0.048% by weight of powder. The three sterols B-sitosterol, campesterol, and sigmasterol were identified. A complete nutrient analysis is also presented. Microbiological analysis was also performed.

References

A glycoprotein (Fucoidan)from Laminaria japonica induces apoptosis (cell death) in HT-29 colon cancer cells

We isolated a novel glycoprotein (Fucoidan) from the brown alga Laminaria japonica that has antiproliferative effects on HT-29 colon cancer cells. We also identified the mechanism by which this glycoprotein, named LJGP, induces apoptosis. MTS assays showed that LJGP inhibited the proliferation of several cancer cell lines (AGS, HepG2, HT-29) in a dose-dependent manner. Especially in HT-29 cells, proliferation was significantly decreased. LJGP treatment on HT-29 displayed several apoptotic features, such as DNA fragmentation, sub-G1 arrest, caspase-3 activation, and PARP degradation. Consistent with sub-G1 arrest, LJGP decreased the expression of Cdk2, cyclin E, cyclin D1, PCNA, E2F-1, and phosphorylated pRb. Furthermore, the increase of p27 expression was observed. We also determined that LJGP-induced apoptosis leads to the formation of a death-induced signaling complex of Fas, FADD, and procaspase-8. LJGP induced the reduction of mitochondrial membrane potential with activation of the Bcl-2 family of proteins and caspase-9. These findings suggest that LJGP inhibits HT-29 cell proliferation by inducing apoptosis, which may be mediated via multiple pathways, including the Fas signaling pathway, the mitochondrial pathway, and cell cycle arrest. **Therefore, LJGP can be a useful treatment option for colon cancer in humans.**

Apoptosis (cell death) induction by glycoprotein isolated from Laminaria japonica is associated with down-regulation of telomerase activity and prostaglandin E2 synthesis in AGS human gastric cancer cells.

Glycoprotein (Fucoidan) isolated from Laminaria japonica (LJGP) is known to exhibit significant cytotoxic activity against human cancer cells; however, the mechanisms of its cytoxicity are poorly understood. In this study, we investigated further possible mechanisms by which LJGP exerts its anti-cancer action in cultured human gastric carcinoma AGS cells. LJGP treatment of AGS cells resulted in inhibition of growth and induction of apoptosis in a time- and concentration-dependent manner, as determined by MTT assay, fluorescence microscopy, and flow cytometry analysis. The increase in apoptosis was associated with up-regulation of pro-apoptotic Bax expression, down-regulation of anti-apoptotic Bcl-2 and IAP family members, and activation of caspase-3 and -9. LJGP treatment markedly down-regulated the activity of telomerase and expression of human telomerase reverse transcriptase, a main determinant of telomerase enzymatic activity, with inhibition of Sp1 and c-Myc expression in a concentration-dependent manner. Furthermore, LJGP treatment also caused a progressive decrease in the expression levels of cyclooxygenase (COX)-2 without significant changes in the levels of COX-1, which was correlated with a decrease in prostaglandin E2 synthesis. **These results provide important new insights into the possible molecular mechanisms of the anti-cancer activity of LJGP.**

Fucoidan-Vitamin C complex suppresses tumor invasion through the basement membrane, with scarce injuries to normal or tumor cells, via decreases in oxidative stress and matrix metalloproteinases

Fucoidan (Fucdn) and vitamin C (VC) were saturatedly dissolved in water and lyophilized and thoroughly ethanol-rinsed until no detection for supernatant vitamin C to form the Fucdn-VC (1:0.23 wt/wt) inclusion body (Fucdn- VC-IB). Fucdn-VC-IB increased not only VC-stabilizing at 37˚C, but also hydroxyl-radical scavenging as shown by ESR/spin-trap method, more markedly than a mere mixture of Fucdn:VC (1:0.23 wt/wt). Invasion of human fibrosarcoma cells HT-1080 through the basement membrane was repressed by Fucdn-VC-IB of non-cytotoxic concentrations without significant inhibition to human skin dermal fibroblasts DUMS-16 cells. Fucdn-VC-IB suppressed the invasiveness-related gelatinases MMP-2/9, and diminished reactive oxygen species inside the cytoplasm around the nucleus, in HT-1080 cells as shown by electrophoretic zymography and the redox indicator NBT assay, respectively. Thus Fucdn-VC-IB markedly exhibits antioxidant and MMP-suppressing activities and preferentially inhibited tumor invasion without cytotoxicity to normal cells, and is suggested as a potent tumor-invasion suppressor. These findings indicate that combination of VC and fucoidan is expected to exert anti-cancer activity more marked than that by treatment with VC or fucoidan alone

References

Fucoidan Induces Apoptosis of Human HS-Sultan Cells Accompanied by Activation of Caspase-3 and Down-Regulation of ERK Pathways

Fucoidan, a sulfated polysaccharide in brown seaweed, was found to inhibit proliferation and induce apoptosis in human lymphoma HS-Sultan cell lines. Fucoidan-induced apoptosis was accompanied by the activation of caspase-3 and was partially prevented by pretreatment with a pan-caspase inhibitor, Z-VAD-FMK. The mitochondrial potential in HS-Sultan cells was decreased 24 hr after treatment with Fucoidan, indicating that Fucoidan induced apoptosis through a mitochondrial pathway. When HS-Sultan was treated with 100 mg/mL Fucoidan for 24 hr, phosphorylation of ERK and GSK markedly decreased. In contrast, phosphorylation of p38 and Akt was not altered by treatment with Fucoidan. L-Selectin and P-selectin are known to be receptors of Fucoidan; however, as HS-Sultan does not express either of these selectins, it is unlikely that Fucoidan induced apoptosis through them in HS-Sultan. The neutralizing antibody, Dreg56, against human L-selectin did not prevent the inhibitory effect of Fucoidan on the proliferation of IM9 and MOLT4 cells, both of which express L-selectin; thus it is possible Fucoidan induced apoptosis though different receptors. **These results demonstrate that Fucoidan has direct anticancer effects on human HS-Sultan cells through caspase and ERK pathways.**

PROMISING ANTIVIRAL (H1N1) GLYCO - MOLECULES FROM AN EDIBLE ALGA (Fucoidan)

From sporophyll of an edible alga Undaria pinnatifida , Fucoidan, a sulfated polysaccharide, was isolated and evaluated in vitro and in vivo as an inhibitor of influenza A virus replication. The Fucoidan showed
in vitro antiviral activity with selectivity index of more than 130. In the time - of - addition experiments, the most sensitive stage of viral replication to the Fucoidan was shown to be earlier than that to a neuraminidase inhibitor oseltamivir. **The binding of the virus to host cells and the penetration into host cells were inhibited by the Fucoidan.**

Fucoidan extracted from Cladosiphon okamuranus Tokida induces apoptosis of human T-cell leukemia virus type 1-infected T-cell lines and primary adult T-cell leukemia cells.

Adult T-cell leukemia (ATL) is caused by human T-cell leukemia virus type 1 (HTLV-1) and remains incurable. The highest endemic area of HTLV-1 carriers in Japan is located in Okinawa, and novel treatments are urgently needed in this area. We extracted Fucoidan, a sulfated polysaccharide, from the brown seaweed Cladosiphon okamuranus Tokida cultivated in Okinawa, Japan and examined its tumor-suppression activity against ATL. Fucoidan significantly inhibited the growth of peripheral blood mononuclear cells of ATL patients and HTLV-1-infected T-cell lines but not that of normal peripheral blood mononuclear cells. Fucoidan induced apoptosis of HTLV-1-infected T-cell lines mediated through downregulation of cellular inhibitor of apoptosis protein-2 and survivin and G1 phase accumulation through the downregulation of cyclin D2, c-myc, and hyperphosphorylated form of the retinoblastoma tumor suppressor protein. Further analysis showed that Fucoidan inactivated NF-kappaB and activator protein-1 and inhibited NF-kappaB-inducible chemokine, C-C chemokine ligand 5 (regulated on activation, normal T expressed and secreted) production, and homotypic cell-cell adhesion of HTLV-1-infected T-cell lines. **In vivo use of Fucoidan resulted in partial inhibition of growth of tumors of an HTLV-1-infected T-cell line transplanted subcutaneously in severe combined immune deficient mice. Our results indicate that Fucoidan is a potentially useful therapeutic agent for patients with ATL.**

References

Apoptosis Inducing Activity of Fucoidan in HCT-15 Colon Carcinoma Cells

The antitumor activity of Fucoidan from Fucus vesiculosus was investigated in human colon carcinoma cells. The crude Fucoidan, a polysaccharide composed predominantly of sulfated fucose, markedly inhibited the growth of HCT-15 cells (human colon carcinoma cells). After HCT-15 cells were treated with Fucoidan, several apoptotic events such as DNA fragmentation, chromatin condensation and increase of the population of sub-G1 hypodiploid cells were observed. In the mechanism of Fucoidan-induced apoptosis, we examined changes in Bcl-2 and Bax protein expression levels and activation of caspases. Fucoidan decreased Bcl-2 expression, whereas the expression of Bax was increased in a time-dependent manner compared to the control. In addition, the active forms of caspase-9 and caspase-3 were increased, and the cleavage of poly(ADP-ribose) polymerase (PARP), a vital substrate of effector caspase, was observed. Furthermore, the induction of apoptosis was also accompanied by a strong activation of extracellular signal-regulated kinase (ERK) and p38 kinase and an inactivation of phosphatidylinositol 3-kinase (PI3K)/Akt in a time-dependent manner. These findings provide evidence demonstrating that the pro-apoptotic effect of Fucoidan is mediated through the activation of ERK, p38 and the blocking of the PI3K/Akt signal pathway in HCT-15 cells. **These data support the hypothesis that Fucoidan may have potential in colon cancer treatment.**

Effect of Fucoidan on the Biotinidase Kinetics in Human Hepatocellular Carcinoma

Background: Hepatocellular carcinoma (HCC) is difficult to treat with anticancer drugs. Therefore, development of new drugs for HCC is required. Materials and Methods: The livers of 14 hepatoma patients accompanied by hepatitis B (2
cases) and hepatitis C (12 cases) were used. The biotinidase kinetics of HCC tissues were compared to those of the adjacent liver tissues of 13 liver cirrhosis (LC) and 1 chronic active hepatitis (CAH). Results: The Kip (the inhibition constant by
biotin) of HCC tissues were consistently higher than those of LC (plus CAH) tissues: the Kip was 450±231 μmol/l in HCC tissues and 240±111 μmol/l in LC (plus CAH) tissues, $p<0.001$. This increase of Kip is considered to be due to an increase of
biotin repulsion by biotinidase in the HCC tissues. Fucoidan, a sulfated poly-fucose, was found to decrease the Kip of biotinidase in HCC tissues, and conversely to increase it in LC tissues. Fucoidan was also found to decrease the Kip of the
hepatoma HuH-6 cells. **Conclusion: These findings suggest that Fucoidan has a potential therapeutic effect on HCC.**

Fucoidan inhibits parainfluenza virus type 2 infection to LLCMK2 cells

The effects of Fucoidan and L-fucose, a fundamental major component of Fucoidan, on the growth of human parainfluenza virus type 2 (hPIV-2) in LLCMK(2) cells were investigated. Fucoidan inhibited cell fusion and hemadsorption, but L-fucose only partly inhibited both. Virus RNA was not detected in the hPIV-2 infected cells cultured with Fucoidan. However, L-fucose did not inhibit virus RNA synthesis. Indirect immunofluorescence study showed that virus protein synthesis was inhibited by Fucoidan, but not by L-fucose. Furthermore, using a recombinant, green fluorescence protein-expressing hPIV-2, it was found that virus entry was inhibited by Fucoidan, but not by L-fucose. **These results suggested that Fucoidan inhibited virus adsorption to the surface of the cells by binding to the cell surface and prevented infection, indicating that the sulfated polysaccharide form was important for the inhibition by Fucoidan.**

References

Antiretroviral Activity of Fucoidans Extracted from the Brown Seaweed Adenocystis utricularis

Treatment of human immunodeficiency virus type 1 (HIV-1, causative agent of AIDS) infection represents a major challenge in antiviral therapeutics. Many difficulties are associated with the treatment, including toxicity, resistance and high costs. Taking this into account, research for novel compounds able to overcome these limitations is needed. Sulfated polysaccharides appear to be interesting, given their abundance as components of seaweeds. Herein, a series of fractions obtained from the brown seaweed Adenocystis utricularis was analysed for in vitro anti-HIV-1 activity. These fractions, which have anti-herpes simplex virus activity, were
determined previously to belong to the family of Fucoidans, sulfated polysaccharides obtained from the cell walls of brown seaweeds. Assays in human PBMC primary cell culture demonstrated that two of the five fractions analysed had potent anti-HIV-1 activity both against WT and drug-resistant HIV-1 strains. For active fractions,
it was also shown that the inhibitory effect was not due to an inactivating effect on the viral particle (i.e. no virucidal activity was detected) but rather to a blockade of early events of viral replication. **Given these encouraging results, these seaweed-derived fractions appear as good candidates for further studies on their potential for in vivo therapy and/or prophylaxis of HIV-1 infection.**

Immunosuppressive Activities of Fucoidan from Laminaria japonica

Effects of Fucoidan from Laminaria japonica on 2,4-dinitrochlorobenzene induced delayed-type hypersensitivity (DTH) reaction and the serum levels of IgG, IgM, complement C3 and C4 were investigated in the present study. Results showed that oral administration of Fucoidan at dose of 150 and 300 mg/(kg" d) for 9 days before the hapten challenge significantly inhibited 2,4-dinitrochlorobenzene induced delayed-type hypersensitivity reaction; and also inhibited the humoral immunity. Serum C3 and C4 levels were markedly reduced by Fucoidan at dose of 150 and 300 rag/kg; and serum IgG and IgM levels were reduced by Fucoidan at dose of 300 mg/kg. **The inhibitory effects of Fucoidan on delayed-type hypersensitivity suggested that it may be potential medication for chronic inflammatory diseases in the future.**

Anti-ulcer effects and biological activities of polysaccharides from marine algae

Fucoidan is a complex sulfated polysaccharide, derived from marine brown algae [17–19], the jelly coat from sea urchin eggs [20–23], and the sea cucumber body wall [23–25]. Most investigations into its biological activity have involved the Fucoidans from brown algae such as Fucus vesiculosus.The Fucoidan from F. vesiculosus mediates a variety of significant biological effects on mammalian cells. Fucus Fucoidan has anticoagulant activity [26–30] and is a potent activator of both anti-thrombin III and heparin cofactor II [30]. Fucoidan inhibits both the initial binding of sperm and subsequent recognition [31]. It also prevents the infection of human cell lines by several enveloped viruses [32,33]. Fucoidan blocks cell-cell binding mediated by P- or L-selectin but not E-selectin [34]. Furthermore, it demonstrates differential binding to interleukins lα and ß, 2, and 6 [35] and hepatocyte growth factor [36]. Since this polysaccharide causes no toxicity or irritation, it may be useful as an anticoagulant, antiviral, anti-inflammatory and contraceptive agent [37–39]. The proposed structure of Fucus Fucoidan consists mainly of 4-sulfated and 2-linked α-fucopyranosyl units [17] and has recently been revised so that the α-fucopyranosyl units are now 1 → 3 linked (Fig. 5) [40]. This structure resembles that determined for a Fucoidan from Ecklonia kurome, another brown seaweed [29,41]. According to most authors, the sulfate groups are linked mainly to the 4-position of the fucose residue [41]. In our previous study, Fucoidan proved effective in healing and preventing of gastric ulcers in experimental animal models on oral administration [42]. Notably, Fucoidan from Cladosiphon okamuranus (Okinawa Mozuku) was more effective in healing ulcers than that from F. vesiculosus. **Furthermore, this Fucoidan blocks both Leb- and sulfatide-mediated adhesion of Helicobacter pylori to gastric cells [43]. Helicobacter pylori are a specific human pathogen. They colonize human gastric epithelium and are linked to serious diseases in the upper gastrointestinal tract, such as gastric and duodenal ulceration and gastric carcinoma [44].**

References

Fucoidan present in brown algae induces apoptosis of human colon cancer cells

Algae induces apoptosis (cell death) of human colon cancer cells. Background: F
sulfated polysaccharide found in brown algae; it has been shown to exhibit a numb
effects, including anti-tumor effects. In this study, we evaluated the effects of Fucoidan on apoptosis in HT-29 and HCT116 human colon cancer cells. Methods: HT-29 and HCT116 cells were cultured with various concentrations of Fucoidan (0 - 20 µg/mL). Apoptosis was assayed via Hoechst staining and Annexin V staining followed by flow cytometric analysis. Western blot analyses and JC-1 staining were conducted to determine the levels of apoptosis-regulating proteins and mitochondrial membrane permeability, respectively. Results: Fucoidan induced substantial reductions in viable cell numbers and apoptosis of HT-29 and HCT116 cells in a dose-dependent manner. In HT-29 cells, Fucoidan also increased the levels of cleaved caspases-8, -9, -7, and -3, and cleaved poly (ADP-ribose) polymerase (PARP) levels. The levels of the X-linked inhibitor of apoptosis protein and survivin were attenuated in the Fucoidan-treated cells. Fucoidan was also shown to enhance mitochondrial membrane permeability, as well as the cytochrome c and Smac/Diablo release from the mitochondria. Fucoidan increased the levels of the Bak and truncated Bid proteins, but reduced the levels of Mcl-1. Additionally, Fucoidan increased the levels of the tumor necrosis factor-related apoptosis-inducing ligand, Fas and death receptor 5 proteins. The caspase-8 and -9 inhibitors Z-IETD-FMK and Z-LEHD-FMK induced a reduction in Fucoidan-mediated apoptosis. Caspase-8 inhibitor inhibited the Fucoidan-induced cleavage of Bid, caspases-9 and -3, and PARP.
Conclusion: The findings of this study indicate that Fucoidan induces apoptosis in HT-29 and HCT116 human colon cancer cells, and that this phenomenon is mediated via both the death receptor-mediated and mitochondria-mediated apoptotic pathways. **These results suggest that Fucoidan may prove useful in the development of a colon cancer-preventive protocol.**

Treatment of human skin with an extract of Fucus vesiculosus changes its thickness and mechanical properties

Recently the researchers found that an extract of Fucus vesiculosus, Fucoidan, which is a type of seaweed, promotes the contraction of fibroblast-populated collagen gels through increased expression of integrin molecules. In this study, they investigated the effects of topical application of an aqueous extract of this alga on the thickness and the mechanical properties of human skin. A gel formulation that included 1% of the extract was applied topically to human cheek skin twice daily for five weeks. A significant decrease in skin thickness measured by B-mode ultrasound was elicited, as was a significant improvement in elasticity measured with a Cutometer as compared with controls. In cheek skin, the thickness normally increases and the elasticity usually decreases with age. **These results suggest that the Fucus vesiculosus extract, Fucoidan, possesses anti-aging activities and should be useful for a variety of cosmetics.**

Fucoidan is the active component of fucus vesiculosus that promotes contraction of fibroblast-populated collagen gels

The fibroblast-populated collagen gel culture method has been evaluated as a dermal model of wound contraction and granulation in tissues during the wound healing process and as an in vitro model of dermal tissue. We previously reported that an extract of Fucus vesiculosus promoted fibroblast-populated collagen gel contraction and that the promotion of the gel contraction was due to the increased expression of integrin alpha2beta1 on the surface of the fibroblasts. In this study, we investigated the active component of the extract of this alga using extraction and fractionation techniques. Water extraction of the alga was followed by precipitation with excess ethanol and then gel filtration with the boundary molecular weight of 30,000. The high molecular weight fraction obtained from gel filtration was fractionated by ion exchange chromatography on diethylaminoethyl cellulose column to give active fractions that have more polar properties. These polar, high molecular weight fractions which contained molecules with fucose and sulfate groups showed significant gel contraction-promoting activity and integrin expression-enhancing activity, and were estimated to be the sulfated-polysaccharide Fucoidan. Commercially available Fucoidan showed similar activities to the above-described fraction of this alga. **Although it remains necessary to precisely identify the specific active component, the above results indicate that Fucoidan is the active component which promotes collagen gel contraction, and also indicate the possibility that it dose so by enhancing the integrin alpha2beta1 expression.**

A 4-week repeated oral dose toxicity study of Fucoidan from the Sporophyll of Undaria pinnatifida in Sprague-Dawley rats

Fucoidan is extracted from brown seaweeds, which can have anti-coagulant, antithrombotic, antitumor, and antiviral activities. However, detailed studies on the toxicology of Fucoidan have not been performed. Here we tested the toxicity of Fucoidan in Sprague-Dawley rats. Fucoidan (1350 mg/kg bw/day for 4 weeks) did not induce statistically significant differences in groups matched by gender with respect to body weight, ophthalmoscopy, urinalysis, hematology, and histopathology. Fucoidan did not change prothrombintime or activated partial thromboplastin time, indicating an inability to change blood clotting. **This study demonstrated that Fucoidan is not toxic under this administration paradigm.**