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Comparison of the efficacy of several nutritional supplements on cancer and normal cell growth

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Introduction

The majority of scientific and epidemiological evidence supports the beneficial effects of micronutrient supplementation on various aspects of health. However, there have also been reports that some dietary supplements and vitamins can promote the growth of cancer. Given that the popularity and use of nutritional supplements is growing, it is important to conduct a comprehensive evaluation of the efficacy of commercially available nutritional supplements.

Nutritional products on the market vary. Many contain single nutrients, while others contain combinations of vitamins, minerals, plant extracts and other nutritional components. The efficacy of such products will differ, but it is rarely, if at all, explained by manufacturers. Usually the selection of specific ingredients is based on published research conducted on individual vitamin, mineral or other nutritional components, however the biological outcome of the combination may depend on the synergistic or antagonistic interactions of its ingredients, the source of its raw materials, and the dose. Manufacturers of nutritional supplements typically do not invest in scientific micronutrient research, mostly taking care of the technological aspect of the formulations.

It is generally accepted that the quality of ingredients (raw materials) used in nutritional supplements is important in assuring the efficacy and safety of the products, as many synthetic ingredients have been shown to be harmful or not effective (1). While some manufacturers promote the use of natural sources in their product's ingredients, they do not provide any evidence that their product demonstrates greater efficacy at the cellular level. As such, in the majority of multi-nutrient supplements, the structure/function claims of a product are backed up by general statements taken from the literature in reference to one or a few ingredients tested individually, or purely by marketing slogans.
In the previous study we showed marked differences in the efficacy of different nutritional supplements marketed in the EU and USA respectively in protecting cells from oxidative stress. This evaluation pointed out the superiority of synergy-based nutrient combinations (2).

In this report we are comparing in vitro efficacy of various supplements on the growth of cancer cells: melanoma cells (A2058), liver cancer cells (HepG2) and normal cells (NHDF). The tested formulas were selected from popular, high-end nutritional supplements sold on the European markets, and nutritional formulas based on nutrient synergy which were scientifically developed at our Institute. The study focuses on the importance of efficacy-testing nutritional supplements, not on the commercial aspects, therefore we anonymised all the products.

Since these products differed not only in nutrient composition, but also in nutrient doses, we compared their efficacy based on the recommended daily intake by the manufacturer of each product, assuming that this reflects the effective dose of a formulation. Products were further divided into two subsets, each based on whether the manufacturers claimed that the ingredients were based on synergy or the nutrient combination was random (or not specified by the manufacturer).
See Table 1.

Table 1: Basic composition of tested nutritional supplement formulas

Product	Contains Vitamins	Contains Minerals	Contains Amino Acids	Contains Bioflavonoids/ Plant Extracts	Contains Active Compounds	Synergy claimed
SetA #1	Yes (3)	Yes (10)	Yes (5)	Yes (2)	Yes (2)	Yes
SetA #2	No	No	No	Yes	No	Yes
SetA #3	Yes (1)	Yes (5)	Yes (4)	Yes (2)	No	Yes
SetA #4	No	No	No	Yes (7)	No	Yes
SetB #1	Yes (13)	Yes (11)	No	Yes (1)	No	No
SetB #2	Yes (13)	Yes (11)	No	Yes (1)	No	No
SetB #3	Yes (13)	Yes (8)	No	Yes (1)	No	No
SetB #4	Yes (13)	Yes (10)	No	Yes (2)	No	No
SetB #5	Yes (13)	Yes (7)	No	Yes (10)	Yes (1)	No
SetB #6	Yes (12)	Yes (9)	No	Yes (1)	Yes (4)	No

Materials and Methods

Cells

Melanoma cells (A2058) were procured from ATCC (American Type Culture Collection, Rockville, MD, USA) and maintained in Dulbecco's Modified Eagle's Medium (DMEM) and supplemented with 10% FBS, 100U/ml penicillin and 100U/ml streptomycin.

HepG2 cells were procured from ATCC (American Type Culture Collection, Rockville, MD, USA) and maintained in Dulbecco's Modified Eagle's Medium (DMEM) and supplemented with 10% FBS, 100U/ml penicillin and 100U/ml streptomycin.

Normal human dermal fibroblasts (NHDF) were supplied by ATCC, maintained in Dulbecco's Modified Eagle's Medium (DMEM) and supplemented with 5% FBS, 100U/ml penicillin and 100U/ml streptomycin, and used in experiments at passages 10th to 12th.

Preparation of supplements for testing

All nutritional supplements were treated identically in accordance with the protocol recommended by Unites States Pharmacopeia (3). Three recommended daily doses of each supplement were powdered (tablets were crushed using ceramic pestle and mortar; capsules were cut open and powder poured out), placed into a glass container with 900 ml of 0.1N hydrochloric acid and incubated for one hour at 37°C in a shaking incubator set with rotation speed of 75 rpm. Resulting solutions were filter-sterilized using 0.2 micrometer pore size filters, aliquoted and kept frozen at-20°C until analyses. Amounts of samples taken for analysis are expressed as number of millionth parts of recommended daily dose of the respective supplement.

Cell viability assay

The assay was performed using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) supplied by Promega, Madison, WI, USA. This is a colorimetric assay for assessing cell metabolic activity. NAD(P)H-dependent cellular oxidoreductase enzymes in viable cells reduce the tetrazolium dye MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to formazan. The concentration of formazan produced is measured by optical density at 490 nm and it is directly proportional to the number of live cells in culture.

Cell morphology

Cells were grown in 48 well plates and subjected to the same conditions as in the cell viability assays and subsequently fixed with methanol and stained with Hematoxylin and Eosin dyes. Cells were observed under a light microscope and representative photos were taken.

Statistical evaluation:

Results are presented as an average of data obtained from triplicate or quadruplicate sets. The set number is specified in each figure. Error bars in each figure represent standard deviation.

Results

The results presented on Fig 1 show that the exposure of melanoma cells to the supplements formulated on the principles of Synergy (Set A) caused a significant reduction of the melanoma cell growth. Supplements A3 and A4 could inhibit melanoma growth completely, while supplement A#1 by about 20%. In contrast, five out of six other commercial products had growth stimulatory effect. Only B#1 showed a pronounced inhibitory effect on melanoma cells. On average B Set formulas stimulated melanoma cell growth by 109%.

As presented in Fig 2, cell growth inhibition by the synergy-based formulas was accompanied by a decrease in cell viability. The exposure of melanoma cells to all Set B formulas did not result in marked changes in cell morphology and viability.

In order to assess whether growth inhibitory and stimulatory effects were limited to a specific cancer cell type we tested the effects of the two representative synergy-based formulas (A#1 and A#3) and five of Set B commercial products on growth of liver cancer cells (HepG2 cells). The results on Fig 3 show that similarly to melanoma cells, the formulas from A and B sets have different effects on the growth of liver cancer cells. Formulas A displayed inhibitory effects on liver cancer cell growth, while all formulas in set B had growth stimulatory effects (on average by 50%).

Subsequently, we compared the effects of the tested formulas on the growth of normal cells. The results on Fig 4 show that formulas in Set A had growth stimulatory effects ranging from 10-50% on normal human skin cells (NHDF) while the majority of formulas in Set B inhibited

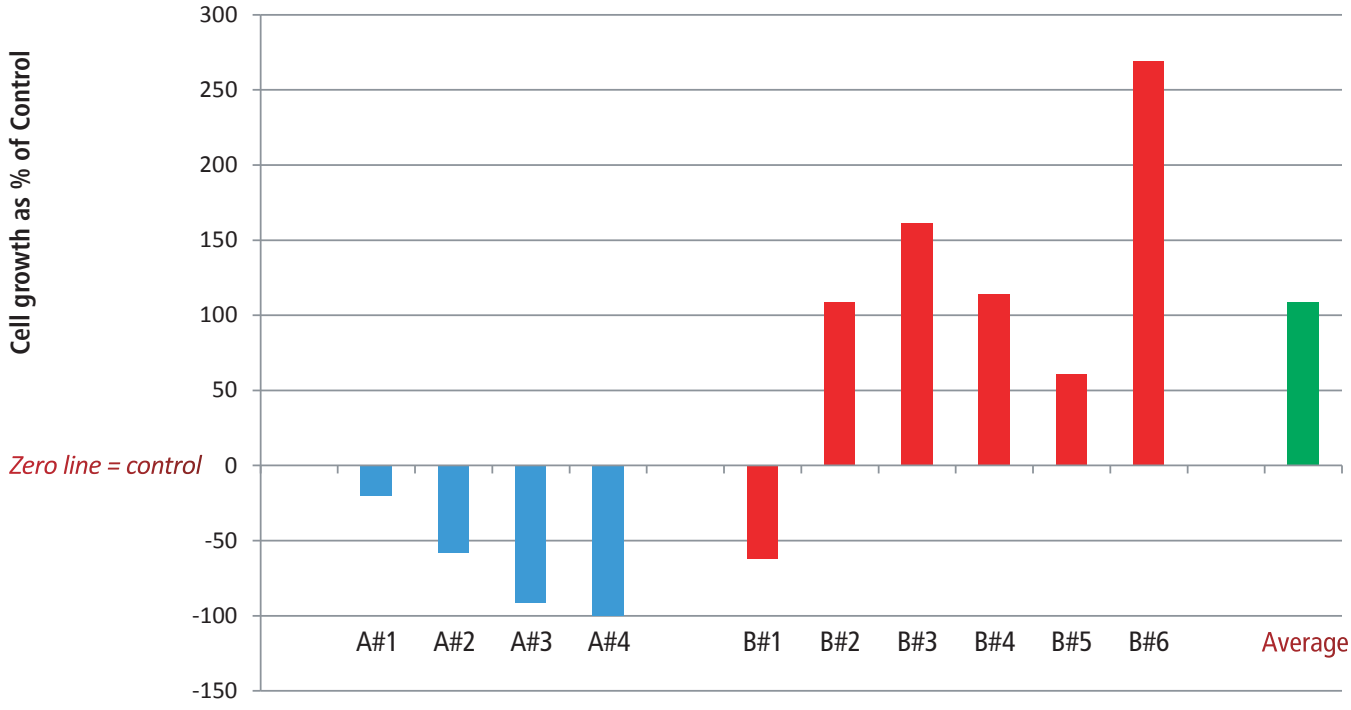

Fig 1. Effects of different nutritional supplements on growth of melanoma cells A2058

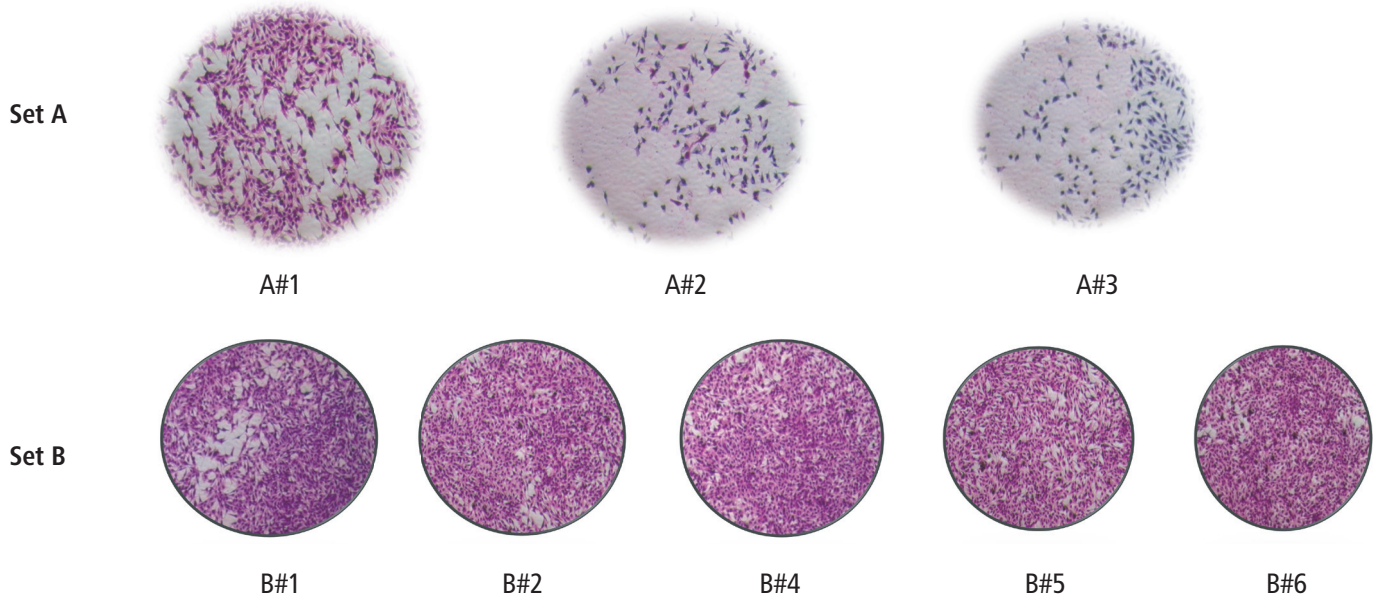

Fig 2. Changes in morphology of melanoma cells A2058 exposed to different nutritional supplement formulas

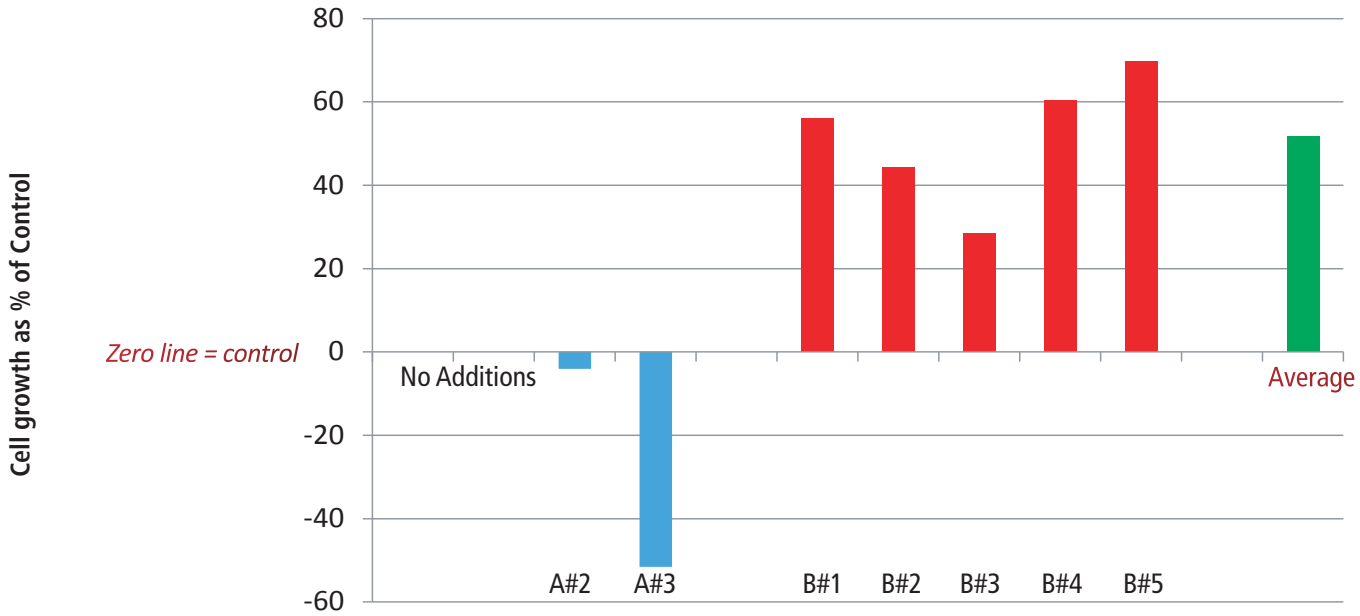

Fig 3. Effects of different nutritional supplements on growth of hepatocarcinoma cells HepG2

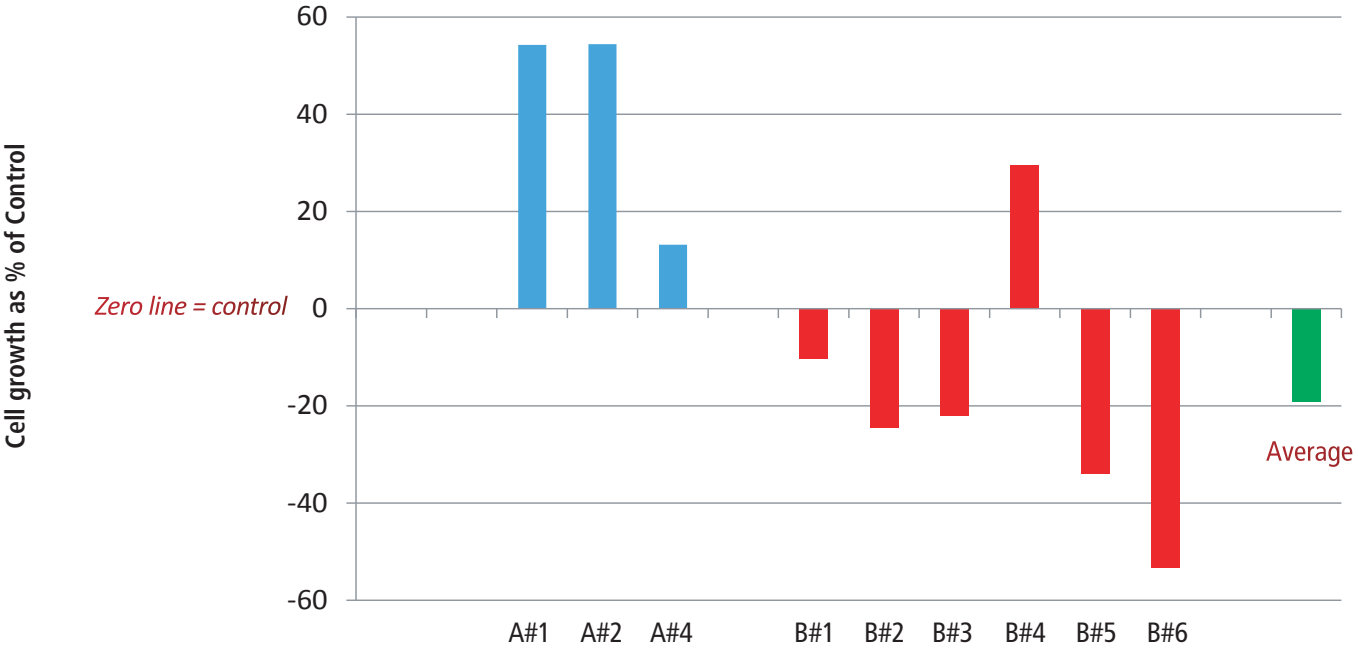

Fig 4. Effects of different nutritional supplements on growth of normal human dermal fibroblast cells

cell growth except for formula B#4. On average B Set formulas inhibited this normal cell growth by about 20%.

Discussion

With the growing popularity of nutritional supplements it is vital to evaluate the safety and efficacy of commercially available products, starting at the cellular level.

The use of supplements varies in different regions of the world. It is especially high in Germany and Denmark (43% and 59% of the adult population respectively take nutritional supplements) and in the US the CDC reports that about 40 percent of the population two months of age and older is taking at least one daily supplement with some taking multiple supplements a day (4). In general, women use supplements more than men do (5, 6, 7, 8, 9).

In the last few years, there have been controversies surrounding the anti-cancer efficacy of certain vitamins and nutritional supplements. Various clinical trials or epidemiological studies have reported positive effects of multivitamin intake against cancer (10, 11), no significant effects (12, 13) or even negative outcomes in people (14). These studies have differed in regard to tested multi-nutrient compositions, sources of ingredients in the formulas, doses, the duration of intake and studied populations.

Therefore in order to eliminate many of these variables we studied the efficacy of nutritional supplements applied directly at the level of cells. The in vitro tests were conducted on melanoma cells (A2058)— a common skin cancer— and hepatocellular carcinoma cells (HepG2). For comparison we also used normal human dermal fibroblast cells, which represent non-malignant healthy cells abundant in the body. Since,

the cellular efficacy of the nutritional combinations may relate to the effective dosage of the products, we made every effort to evaluate these widely variable products by applying the same uniform standard. As such, all formulas were solubilised according to the method recommended by the US Pharmacopeia and they were applied according to the doses stated on the product label. The study did not assess the sources of raw ingredients in the formulas.

We attribute the consistent anti-cancer effects of synergy-based formulations to the fact that these formulas were subjected to scientific studies and contain carefully selected sources of raw materials. This deliberate selection of ingredients and scientific testing could be linked to their recommended daily dose exhibiting greater safety and efficacy at the cellular level.

The results imply that the composition and selection of ingredients as well as correct dosing is vital for achieving cellular efficacy and safety of a final formula. Therefore sellers who don't perform adequate testing of not only individual ingredients but also their entire formulations can contribute to the presence of ineffective products on the market. This not only prevents people from achieving the health benefits of nutrient supplementation, but also in the longer term undermines the credibility of the nutritional supplement industry as a whole, making people justifiably refrain from taking nutritional supplements.

We therefore propose that our testing and deliberate formulation based on the principles of synergy is important not only for people who take our products, but for the overall health industry.

This is a limited study for research purposes and does not take into account batch to batch variations of any of the tested products. It is possible that results can vary with different cell types and conditions.

Conclusions:

The results of this in vitro study indicate that multivitamins and antioxidant-containing supplements differ in affecting cancer and normal cell growth. In general, the formulations based on synergistic nutrient composition and which have been scientifically developed can significantly inhibit the growth of cancer cells, while five out of six of the commercially available formulas tested in this study displayed different degrees of cancer cell growth stimulation. The results stress the importance of scientifically testing nutritional supplements before making them available to the general public.

Acknowledgments:

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