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MICRONUTRIENT SYNERGY IN CONTROLLING KEY MECHANISMS OF CANCER

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Introduction

Cancer is an abnormal and uncontrolled growth and spread of transformed cells, which due to their genetic defect became immortalized. It is the most feared malady and the second leading cause of death in the Western world, affecting people of all ages. Almost 1.4 million new cases of cancer are diagnosed each year. Despite \$25 billion dollars spent on cancer research over the past 20 years, the death rate from cancer has increased and a cure is not even in sight.

There are more than 100 different types of this disease which can affect virtually all organs and tissues. Cancer can originate almost anywhere in the body and then spread to other parts, a process which is called metastasis. The risk of developing cancer during one's lifetime is 50% for men and 33% for women. The most prevalent type of cancer in men is prostate cancer and in women, breast cancer. In both genders the most dominant types are lung and colorectal cancers. Leukemia (blood cancer) is very common in children. Cancer thus represents a tremendous burden on patients, family and society.

Hallmarks of Cancer

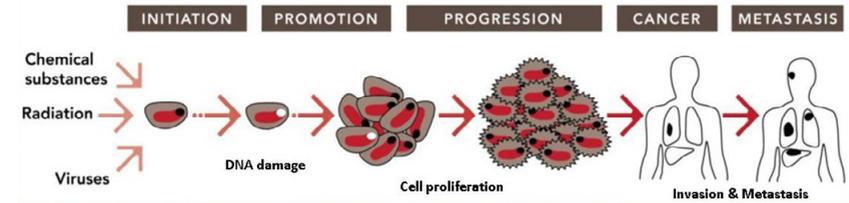
The pathological criteria for identifying cancer are.

- Continuous cell proliferation and tumor growth
- Invasion (incursion of tumor cells into adjacent tissue/migration)
- Metastasis (spread through the blood stream and lymphatic system to other organs)
- Angiogenesis (formation of new blood vessels in tumors)
- Defect in Apoptosis (programmed cell death)

Effective control of even one of the above parameters can bring cancer progression and metastasis into a halt.

The Origin of Cancer

- Chemical substances: Environment, food, air, water and occupational exposure
- Radiation: Sunlight, X-ray
- Viruses (RNA and DNA): i.e. Epstein-Barr, Hepatitis B&C, Human papilloma

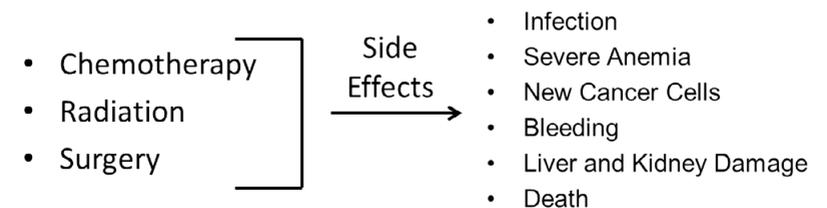


Radiation, chemicals and viruses have been recognized as cancer-causing agents in human and many animal species. Although there is a great diversity in the nature of these agents, the end cellular response is always the same - the production of cancer cells.

The transformation of normal cells to cancerous cells is a complex process and occurs through three distinct phases including initiation, promotion and progression. Initially these agents react with the DNA of the cell and cause its alteration or damage. These altered or damaged cells propagate to form pre-neoplastic cells and finally turn to malignant or cancerous cells. In an advanced cancer the cells escape from a tumor mass and metastasize by spreading through the blood stream and lymphatic system to other parts of the body such as the brain, liver, kidneys, lungs or bones,

Standard Cancer Treatments and Side Effects

Billions of dollars have been spent on cancer research to determine the cause and to find a cure for cancer. Despite the expenditure, death from cancer has actually increased. Although each treatment offers short lasting and limited benefits, it does not cure cancer and results in compromising the quality of life of a patient. The choice of the therapy primarily depends on the type and stage of cancer.



For decades the mainstay of cancer treatment has been surgery, chemotherapy and radiation.

Surgery: Surgical excision of a tumor is used to remove tumors and surrounding tissues to ensure that cancer cells do not remain in the area. But surgery can't be used if multiple tumors are present, nor can it be used in especially hard to access places. Even if surgery is successful the cancer cells may not be completely removed from the affected area of the tissue, leaving some behind. The remaining cells start to proliferate, resulting in returned malignancy and more cancers.

Radiation: radiation therapy involves the use of x-rays. This is damaging to normal tissues, cellular DNA and the immune system, triggering new cancers.

Chemotherapy: chemotherapy involves the use of powerful toxic chemicals that are directly injected into the blood stream to destroy cancer cells. These agents do not distinguish between cancer cells and healthy cells, so a good number of healthy cells are destroyed, especially frequently dividing cells such as bone marrow cells leading to severe anemia, cells lining gastrointestinal track causing malabsorption, diarrhea, nausea, vomiting, intestinal bleeding, hair follicles causing hair loss and the immune cells resulting in impaired immunity, infections, weakness, and fatigue.

Moreover the cellular damage caused by chemotherapy and radiation involves destruction of the body's connective tissue, facilitating spread of cancer, infections, and bleeding. Chemotherapy agents can also cause kidney and liver damage, trigger new cancers, facilitate metastasis thereby drastically reducing the survival of the patient, and finally death. Chemotherapy has been associated with poor outcomes secondary to its severe toxicity, immune involvement, and genotoxicity giving rise to new cancers, as well as development of drug resistance.

Scientific review of clinical trials conducted between 1990 and 2004 on 22 types of cancer has shown that chemotherapy has merely increased 5-year survival rate by 2.1% and was not effective at all in several types of cancers (Med. Oncology 2004; 16, 549-560). Unfortunately the cancer reappears despite of treatments thereby drastically reducing the survival rate. A permanent cure is not even in sight.

Furthermore, standard cancer treatments are extremely costly and have led to the sky-rocketing cost of healthcare. The only beneficiary of this situation is the pharmaceutical industry which makes tremendous profits from the sales of its cancer/chemotherapy drugs but also on a multitude of others drugs and procedures needed to control severe side effects of its cancer 'treatment'.

Cellular Medicine Approach

A lack of progress in developing effective treatments for cancer in the last decades demands a revision of the current approaches and the development of new strategies in the treatment of cancer. Therefore it is not surprising that 80% of cancer patients seek alternative therapies. In the search for an effective solution to cancer, the work of Dr. Rath and his colleagues, based on the Cellular Medicine approach and nutrient synergy, brings a new perspective of a natural control of cancer.

Goal: Stopping cancer invasion and metastasis. Metastasis is responsible for 90% of deaths from cancer.

Key physiological target: Increasing stability and integrity of ECM and connective tissue as a natural barrier for cancer cells spread.

Approach: Inhibition of ECM digestive enzymes (MMPs, uPA) together with optimizing collagen synthesis and structure as a scaffold for assembling healthy ECM and connective tissue.

Applied components: Select natural compounds working in biological synergy defined as Nutrient Synergy (NS).

Nutritional Synergy effects: Natural substances affecting various defined metabolic pathways simultaneously allow for attacking multiple mechanisms of cancer at once decreasing a likelihood of the treatment resistance or escape by activating alternative biological mechanisms.



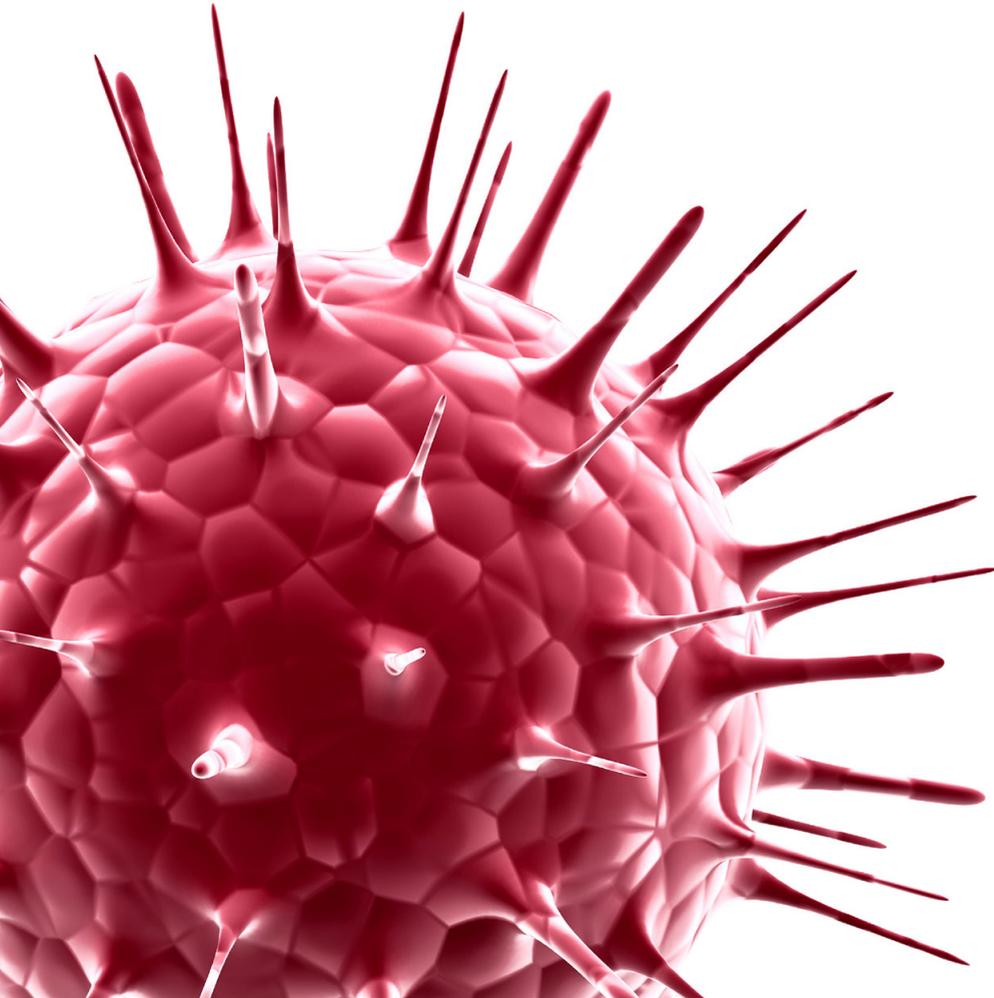
| Micronutrient Synergy | |
|-----------------------------|----------------------------|
| <u>Vitamins</u> | <u>Minerals</u> |
| • Vitamin C | • Selenium |
| <u>Amino Acids</u> | • Copper |
| • L-Lysine | • Manganese |
| • L-Proline | <u>Polyphenols</u> |
| • L-Arginine | • Green Tea Extract (EGCG) |
| • N-Acetyl L-Cysteine (NAC) | • Quercetin |

Efficacy of Nutrient Synergy against Cancer

The synergy based micronutrient synergy (NS) was applied in the in vitro and in vivo studies using various cancer cell lines to test its effects on the key mechanisms of cancer, including tumor cell proliferation, growth, invasion, metastasis, angiogenesis and apoptosis.

NS was found to be effective in controlling the mechanisms of cancer listed below at once.

- Inhibition of tumor growth/proliferation
- Inhibition of invasion
- Inhibition of metastasis
- Inhibition of angiogenesis
- Induction of apoptosis



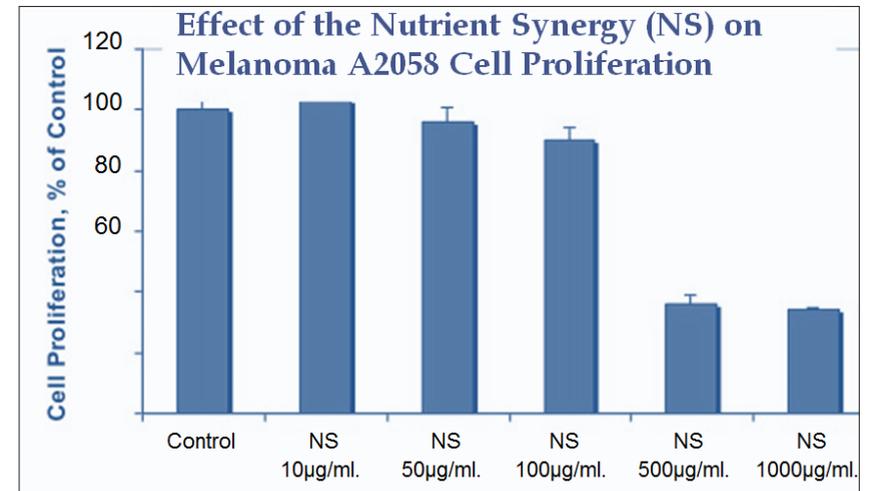
Experimental Results

Proliferation of Cancer Cells

Continuous proliferation (growth) of cancer cells leads to the formation of tumors. We evaluated the effect of NS on this process using various cancer cell lines in vitro. Forty-five different human cancer lines were selected on the basis of organ malignancies that included carcinomas, sarcomas, leukemia and five murine cancer cell lines.

• NS Inhibits the Multiplication of Cancer Cells in vitro

The effect of NS on growth of human melanoma cells (A 2058,) is shown in Figure 1. NS had a slight inhibitory effect on the growth of these cancer cells, when used at concentrations of 10 and 50 µg/ml. More potent effect resulting in about 70% inhibition of cell proliferation was observed at NS concentrations of 500 and 1000 µg/ml.



The inhibitory effect of NS on cancer cells proliferation was confirmed in a variety of human cancer cell lines as presented in Table 1.

Table 1

| Cancer cell line | % Inhibition of cell growth at NS 1000 µg/ml | Cancer cell line | % Inhibition of cell growth at NS 1000 µg/ml |
|--------------------------|--|-----------------------------------|--|
| Prostate LNCaP | 80% | Chondrosarcoma SW-1353 | 39% |
| Melanoma A2058 | 64% | Pancreas MIA PACA-2 | 38% |
| Liposarcoma SW-872 | 61% | Cervical cancer HeLa | 37% |
| Osteosarcoma U2OS | 60% | Fibrosarcoma HT1080 | 37% |
| Glioma A-172 | 50% | Hepatocellular carcinoma SK-Hep-1 | 33% |
| Breast MBA-MB-231 | 50% | Synovial carcinoma | 28% |
| Prostate DU-145 | 47% | Rhabdomyosarcoma | 20% |
| Cervical Cancer DoTc2451 | 45% | Lung carcinoma A-549 | 20% |
| Prostate PC-3 | 40% | Osteosarcoma MNNG | 10% |

• NS Inhibits Tumor Growth in vivo.

Our findings showing that NS can inhibit cancer cell growth in vitro were confirmed in the experiments in vivo.

In a typical experiment tumors were induced in female athymic (nude) mice by injecting subcutaneously 2x10⁶ cervical cancer cells HeLa (xenograph method). Following the injection the mice were randomly divided into two groups: One group was fed regular mouse chow and the other group was fed the same diet but supplemented with 0.5% NS. After four weeks, the mice were sacrificed and their tumors were excised, weighed, and processed for histology.

We observed that dietary intake of NS resulted in 59% inhibition of the weight of cervical cancer (HeLa) tumors compared to controls (Fig 2). The histology of the tumors confirmed a significant decrease in the proliferation marker Ki67 in tumors originating from NS supplemented mice as indicated by the immunohistochemical analysis (Fig.3)

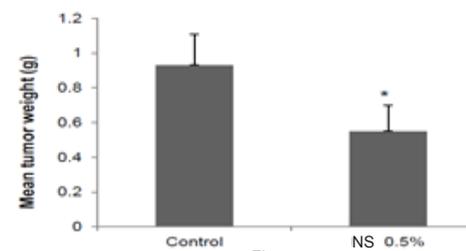


Figure 2

The dietary intake of NS inhibited growth of cervical tumors (HeLa) by 59% compared to control

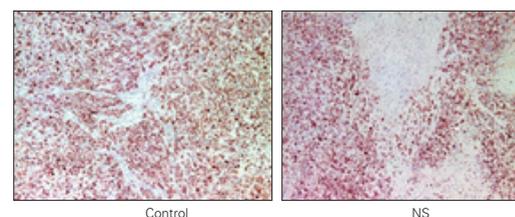


Figure 3

Tumors developed in NS supplemented mice had significantly decreased the proliferation marker Ki67 as indicated by immunochemical analysis of the tumor tissues

Xenograft cancer studies in nude mice were also conducted using a variety of other cancer cell lines. The results in Table 2 show the efficacy of NS in the reduction of tumor growth in various types of cancers.

Table 2

| Cancer Cell Line | Reduction in tumor weight by NS supplemented diet over control diet |
|----------------------------------|---|
| Colon Cancer cells (HCT-116) | 63% |
| Melanoma Cells (A2058) | 57% |
| Prostate Cancer Cells (PC-3) | 53% |
| Osteosarcoma Cells (MNNG) | 53% |
| Breast Cancer cells (MDA-MB-231) | 27% |
| Neuroblastoma Cells | 25% |

• NS Inhibits Growth of Chemically Induced Tumors

Based on above encouraging results we conducted in vivo study testing the efficacy of NS in inhibiting the development of cancers induced by various chemicals and affecting different organs.

Accordingly, we studied the effect of NS on carcinogenic process induced by N-methyl-N-nitrosourea (MNU), in the mammary glands in rats as well as the efficacy of NS on the development of lung cancer in mice exposed to urethane and skin cancer induced by DMBA (Dimethyl benz-anthracin).

A. Chemically-Induced Mammary Tumors

NS inhibited N-methyl-N-nitrosourea-induced (MNU) mammary tumors

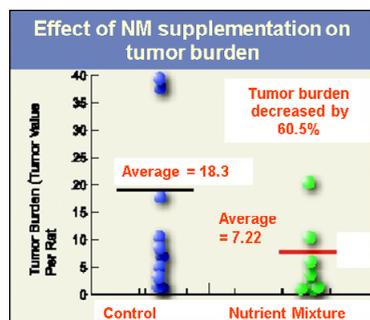


Figure 4

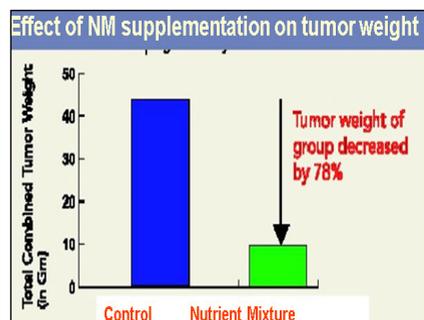


Figure 5

Breast tumors were induced in female rats by exposing them to N-methyl-N-nitrosourea (MNU). We observed that mice fed a diet enriched with NS developed fewer tumors as only 6 tumors developed in these mice compared to 19 tumors in the control. In addition, the tumors which developed in the NS supplemented group of mice had significantly decreased weight by 78% and tumor burden by 60.5%. (Fig 4, Fig 5)

B. Chemically Induced Lung & Skin Tumors

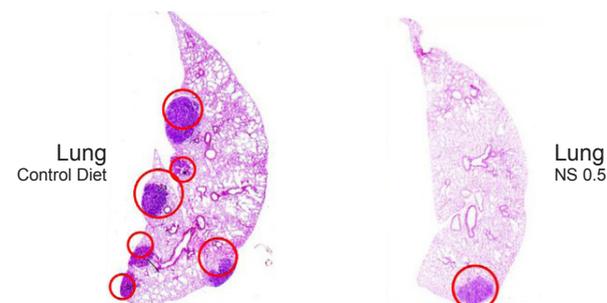


Figure 6



Figure 7

After the exposure to Urethane mice were divided into two groups: one was fed a control diet, the other group was placed on a diet supplemented with 0.5% NS. Mice in both groups developed tumors in the lungs, however mice fed a diet enriched in NS developed only a few tumors (Fig 6).

Similarly there was a large number of skin tumors (papilloma) developed in the DMBA exposed mice fed control diet. In contrast, there was only limited number of skin tumors in the NS supplemented mice (Fig 7).

The results from these in vivo cancer studies clearly indicate that NS could inhibit cancer development in three different organs induced by three different chemical carcinogens.

Invasion of Cancer Cells

Cancer is capable of spreading through the body by two mechanisms: invasion and metastasis. Invasion refers to the direct migration and penetration of cancer cells into the neighboring tissues. We studied the effects of NS on cancer cells invasion through extracellular matrix and their migration capability using in vitro approach.

• NS Can Stop Invasion of Cancer Cells through Extracellular Matrix (Matrigel) In Vitro

This picture illustrates a typical invasion experiment on the example of fibrosarcoma cells HT-1080. The invasion studies were conducted using Matrigel, which is an extracellular matrix-coated vial insert routinely used for cell invasion assays. The cells are placed on top of the specific vial inserts and are exposed to different tested agents, such as in our experiments, the different concentrations of NS. After 24 hours, the number of cells that could destroy the extracellular matrix and cross the Matrigel barrier was examined under the microscope and counted. (Fig 8A and 8B)

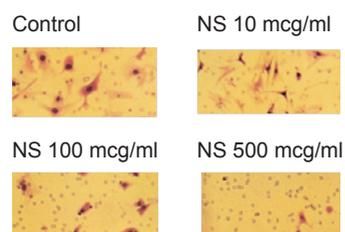
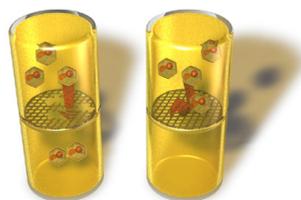


Figure 8A

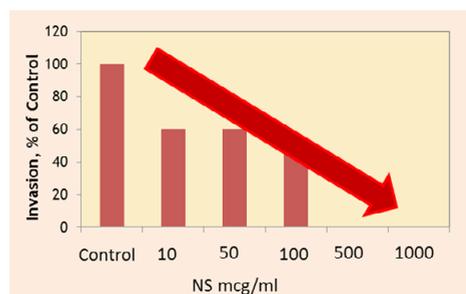


Figure 8B

The results show that in the presence of increasing concentrations of NS (10 mcg/ml, 100 mcg/ml, 200 mcg/ml and 1000 mcg/ml) the invasion of melanoma cells through Matrigel was inhibited by 40%, 50%, 70% and 100% respectively.

Table 3 INHIBITION OF INVASION

| Human Cancer Cell Line | NS Required for 100% Inhibition of Invasion |
|-----------------------------|---|
| Breast cancer (MDA-MB231) | 100 mcg/ml |
| Breast cancer (MCF7) | 100 mcg/ml |
| Osteosarcoma (MMNG) | 100 mcg/ml |
| Pancreatic cancer (MIAPaCa) | 500 mcg/ml |
| Prostate cancer (LNCaP) | 500 mcg/ml |
| Cervical cancer (CCL2) | 500 mcg/ml |
| Lung carcinoma (A549) | 500 mcg/ml |
| Testis cancer (NT2/DT) | 500 mcg/ml |
| Synovial carcinoma | 1000 mcg/ml |
| Fibrosarcoma (HT1080) | 1000 mcg/ml |
| Prostate cancer (PC3) | 1000 mcg/ml |
| Ovarian cancer (SKOV3) | 1000 mcg/ml |
| Renal carcinoma (796-0) | 1000 mcg/ml |
| Bladder cancer (T24) | 1000 mcg/ml |

The exposure to NS resulted in the complete inhibition of the invasion of all human cancer cell lines tested so far (50 in total) in a dose-dependent manner, which indicates strong anti-invasive cancer properties of NS. The results for select cancer cell types are shown in Table 3

• NS can inhibit migration of cancer cells, in vitro

In addition to its anti-invasive properties, the exposure of cancer cells to NS reduced their migration capabilities. This is shown in Fig. 9 on the example of fibrosarcoma HT-1080 cells.

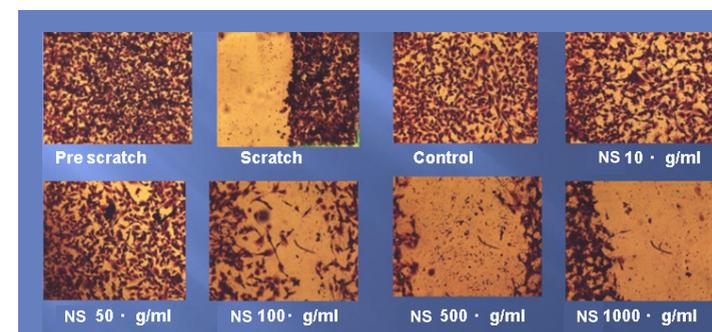


Figure 9

Cancer cells were growing on specific plates until they were near confluent and then a 2-mm wide single uninterrupted scratch was made from top to bottom of the plate opening a wide empty area for cell migration. Subsequently the cells on the plates were exposed to different concentrations of NS for 24 hours (Fig. 9). The results show that the migration of these cancer cells could be inhibited in the presence of increasing NS concentrations. Similar results were obtained using other cancer cell types.

Cancer Metastasis

Metastasis refers to the ability of cancer cells to separate from a tumor mass, penetrate into blood vessels and the lymphatic system, and circulate via the blood stream to reach to distal body organs, where they start a new growth forming separate tumors. It is the most dangerous weapon of cancer as 9 out of 10 people with cancer die not of primary tumors but of metastasis. Conventional medicine approaches are not effective in permanently stopping metastasis and there is no cure for it.

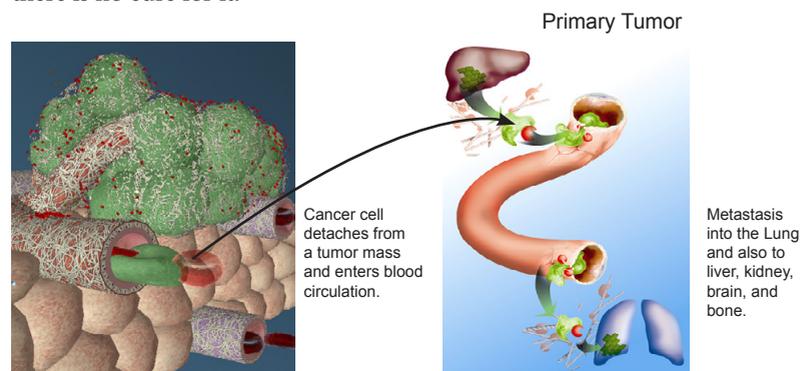


Figure 10

Having demonstrated earlier that NS could inhibit invasion and migration of tumor cells, we conducted a series of studies to determine whether NS can inhibit metastasis of cancer. We tested the efficacy of NS in various processes that directly or indirectly affect the ability of cancer cells to metastasize using both *in vivo* and *in vitro* approaches.

• NS Inhibits Lung, Liver & Spleen Metastasis *in vivo*

Melanoma B16F0 cancer cells were injected via the tail vein in C57BL/6 female mice. We observed that pulmonary metastasis of melanoma was reduced by 63% in mice supplemented with the NS in their diet. By providing the NS intravenously, the inhibitory effect of NS on cancer metastasis to the lungs was even higher and reached 86% (Fig.11A).

We also investigated the effect of NS on metastasis of melanoma to other organs. After injection of melanoma cells directly into the spleen of the mice, the animals were divided into two dietary groups: a standard diet group and NS supplemented group. The mice receiving NS in their diet showed decreased tumor growth in the spleen compared to the control mice and also reduced me-

tastasis to the liver. In addition, hepatic metastasis in NS supplemented mice was reduced by 55% compared to the control (based on mean liver weights of the group) (Fig.11B and 11C)

• NS Positively Affects Stability and Integrity of Extracellular Matrix as a Key Factor in Effective Control of Cancer Metastasis

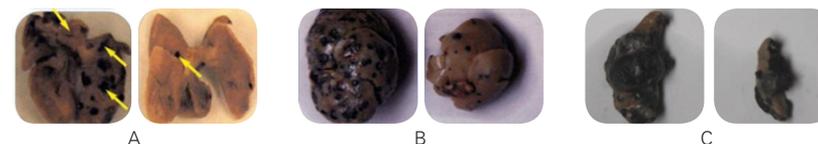


Figure 11

Extracellular matrix and basement membranes form a natural barrier that can constrain cancer cells thereby controlling their tissue invasion and metastasis. Therefore our NS synergy components include select natural compounds that are critical in supporting optimum collagen and extracellular matrix synthesis and have inhibitory effects on the specific enzymes which destroy collagen and decrease integrity of extracellular matrix in the tissue.

Therefore, we studied the efficacy of NS in controlling the enzymatic activity of the key enzymes involved in extracellular matrix destruction cascade in cancer (MMPs and uPA) and on the synthesis of collagen and other extracellular matrix components.

• NS inhibits the secretion MMP-2 and MMP-9 in cancer cells

Metastatic potential and invasiveness of cancer are attributed to the increased activity of matrix metalloproteinases (MMPs), which are a class of zinc-dependent proteinases. Among various enzymes in this class, the secretion of MMP-2 and MMP-9 has been correlated with the aggressiveness of tumor growth and the invasiveness of cancer. Elevated levels of these MMPs have been associated with poor prognosis in various types of human cancers.

We studied the effect of NS on MMP2 and MMP9 secretion in several different cancer cell lines based on organ malignancies, including carcinomas, sarcomas and leukemia. Based on the pattern of MMP-2 and MMP-9 secretion, the various cancer cell lines could be categorized into three groups:

- 1) secreting only MMP-2;
- 2) secreting MMP-9 only; and
- 3) secreting both MMP-2 and MMP-9.

Secretion of MMPs can be measured using Zymography assay which is based on a separation of these proteins by gel electrophoresis and detecting the presence of MMP2 and MMP9 based on their proteolytic activity. The MMPs are seen as glowing bands and their intensity can reflect different levels of the enzyme. Our results have demonstrated that NS can inhibit the expression of MMPs in all three different classes of cancer cells in a dose-dependent manner.

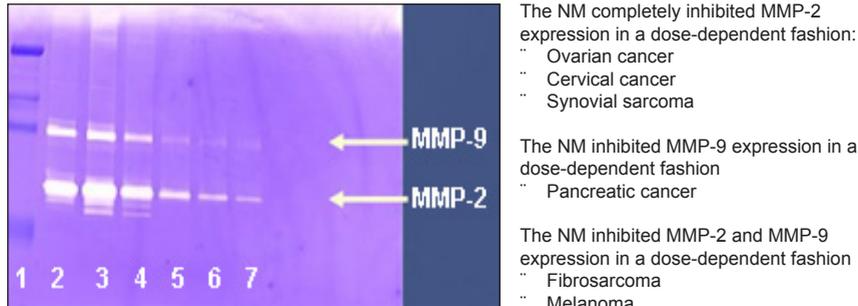


Figure 12

The example of fibrosarcoma HT-1080 cells, a representative of a cell line that secretes both MMPs, shows that NS can inhibit the secretion of MMP9 and MMP2 by these cells in a dose dependent manner (shown in Figure 12).

The inhibitory effect of NS on MMPs secretion was confirmed in the in vivo studies, which showed that the tumors developed in animals fed NS supplemented diets display a significantly lower MMPs activity compared to controls.

• NS Increases the Expression of Tissue Inhibitors of Matrix Metalloproteinases (TIMPs) in vitro

Tissue inhibitors of metalloproteinases (TIMPs) play a critical role in the homeostasis of extracellular matrix (ECM) by regulating the activity of extracellular matrix digesting enzymes, the MMPs. TIMPs are well-known for their ability to inhibit MMP activity thereby inhibiting tumor growth and metastasis. However, many studies suggest that TIMPs are multifunctional proteins, which can also regulate cell proliferation, apoptosis, proMMP-2 activation, and angiogenesis.

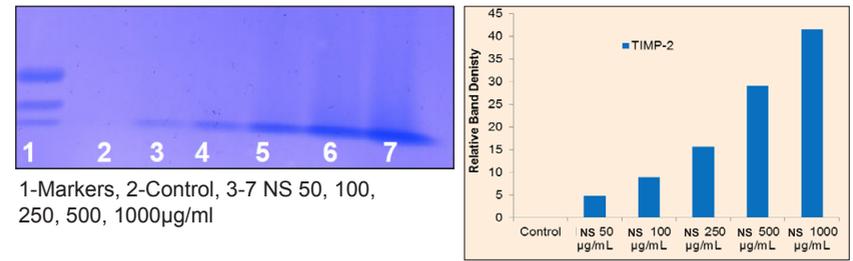


Figure 13A

Figure 13B

The exposure of prostate cancer cells (DU-145) to NS resulted in an increased expression of a natural inhibitor of metalloproteinase (TIMP-2) in a dose dependent manner (Fig. 13A and 13B). The similar effect of NS was confirmed in 25 different human cancer cell lines.

• NS Inhibits Expression of the Urokinase Plasmin Activator (u-PA) in vitro

Urokinase plasmin activator (uPA) also called Urokinase is a proteolytic enzyme, which activates the conversion of plasminogen to the protease plasmin. Activation of plasmin triggers a proteolysis cascade affecting thrombolysis and extracellular matrix degradation. Elevated levels of urokinase and several other components of the plasminogen activation system are found to be correlated with tumor malignancy. It has been shown that the tissue degradation following plasminogen activation facilitates tissue invasion and, thus, contributes to metastasis. Higher activity of uPA has been also associated with angiogenesis.

NS inhibited u-PA expression of various cancer cell lines, including prostate DU-145, as shown in Fig.14A and 14B. -Similar inhibitory effect of NS on uPA expression was observed in 25 different types of human cancer cell lines.

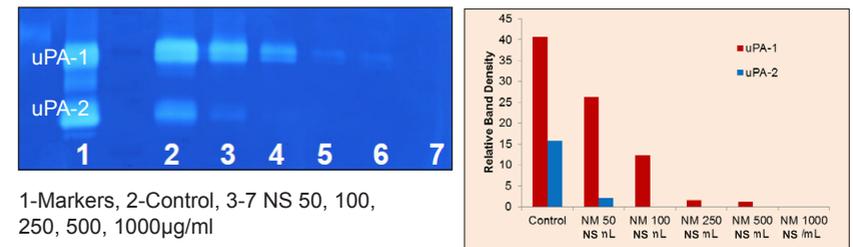
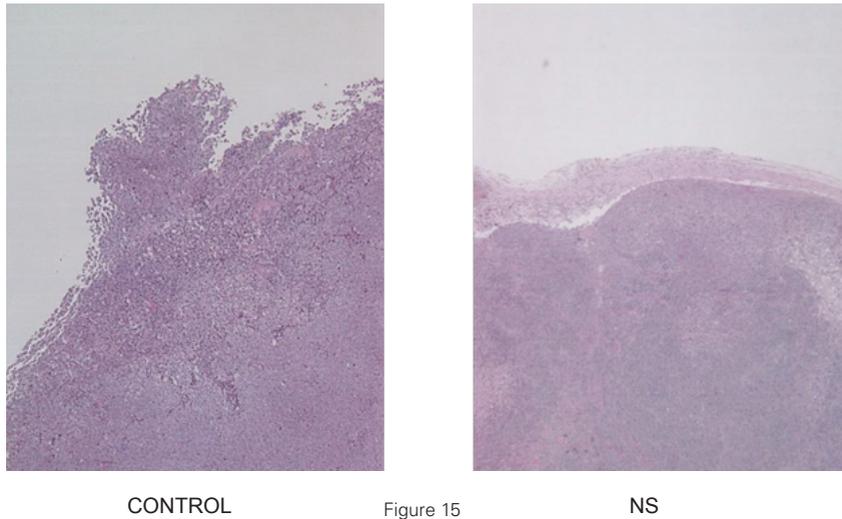


Figure 14A

Figure 14B

• NS Affects the Synthesis of Extracellular Matrix Components

We studied the effect of NS on the synthesis of important constituents of extracellular matrix that are involved in tumor development and progression.



The results of our studies show that NS dietary supplementation affects the formation of a connective tissue border surrounding tumors. This is shown using the example of Fibrosarcoma cancer. The cross-section of a tumor developed in the NS supplemented animal shows a defined collagenous border compared to the tumor from an animal fed control diet. Such a fibrous border can form a natural barrier against cancer cell invasion into an adjacent tissue.

We observed earlier that a special strain of mice (GULO^{-/-}), which like humans lost an ability to produce vitamin C internally, also develop tumors that do not have well defined fibrous borders. Adding vitamin C to the diet of these animals resulted in an increased collagen production and the formation of a dense tumor border accompanied by impaired tumor growth and metastasis.

• NS Affects Collagen I, Collagen IV and Fibronectin in Tumors

Our study on the development of cervical cancer (HeLa) in mice has shown that the tumors developed in mice fed a diet supplemented with NS are not only smaller, but also have more dense connective tissue. This is demonstrated on an immunohistology slide from the tumor, which shows an abundance of collagen I, IV (base membrane collagen), and fibronectin compared with the tumor developed in animals fed control diet. (Fig.16)

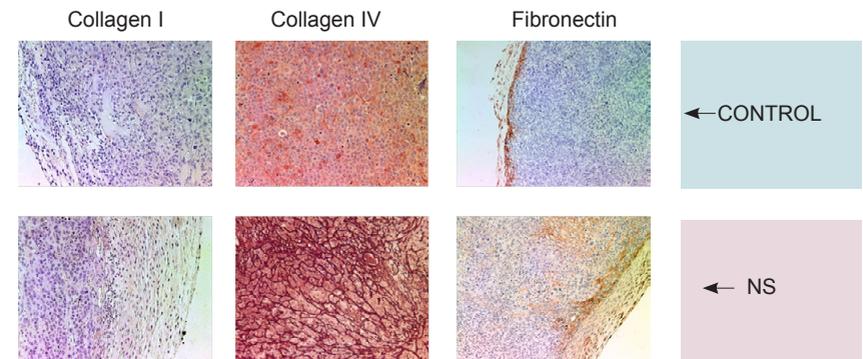


Figure 16

Angiogenesis

Angiogenesis is the formation of new capillaries from the existing blood vessels. It is considered to be a fundamental process in physiological and pathological conditions. Angiogenesis is necessary for tumor growth, invasion and metastasis. It not only allows the tumor to increase in size, but also provides a route for metastasis to distal sites in the body. A tumor mass less than 0.5 mm in diameter can receive oxygen and nutrients by diffusion, however, any increase in tumor mass beyond this requires new blood vessels to support tumor growth.

Formation of new blood vessels is a complex process involving interplay of various growth factors, intra and extracellular signals but also enzymatic destruction of connective tissue to facilitate the formation and expansion of blood capillaries into the tissue.

• NS Affects Various Aspects Involved in Tumor Angiogenesis

Based on our studies which have shown that NS can inhibit the secretion of MMPs, uPA and curtail tumor growth, invasion and metastasis, we tested the effects of NS on angiogenesis. We used several in vitro and in vivo experimental models to study angiogenesis:

In vivo

- Histological assessment of the vascularization of tumors induced by subcutaneous injection of cells (MNNG xenograft) in nude mice and expression of angiogenesis promoting factor (VEGF) in these tumors
- New blood vessel formation in chicken embryos (CAM assay)
- Formation of capillary tubes by human umbilical cord endothelial cells (HUVEC)

In vitro

- Expression of pro-angiogenic factors such as VEGF, angiopoietin-2, bFGF, PDGF and TGF- β 1 by U2OS cells

Tumor Vascularization in vivo. Xenograft studies in nude mice using human osteosarcoma MNNG-HOS cells showed that the NS supplemented mice developed not only smaller tumors by 53% compared to control mice, but also these tumors were less vascular (Fig.17).

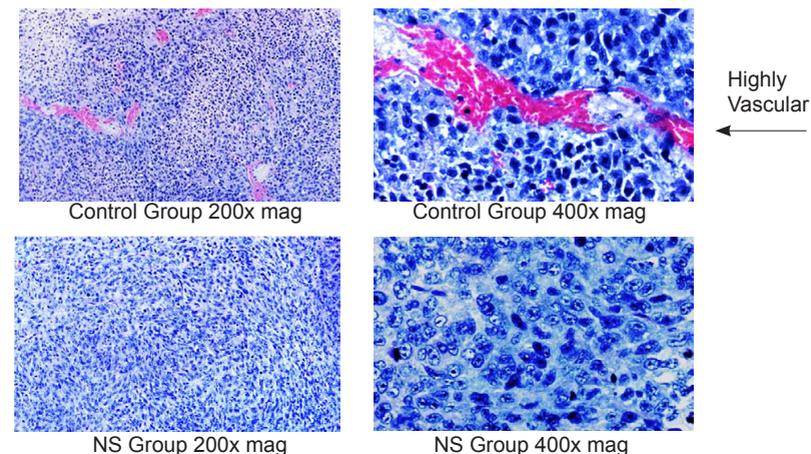


Figure 17

New Blood Vessels Formation in vivo: The chorioallantoic membrane (CAM) assay in chick embryos and other in vivo assays demonstrated a significant antiangiogenic activity of NS. We induced the formation of new blood vessels in chicken embryos by exposing them to bFGF (basic fibroblast growth factor), a well-known inducer of angiogenesis. Subsequently, the embryos were exposed to increased concentrations of nutrient mixture. The results in Fig 20 show a significant reduction of blood vessel branch points from 22 (in bFGF only exposed embryos) to 10 in the embryos with added NS. The number of blood vessel branch points is relative to the number of newly sprouting blood capillaries (Fig. 18).

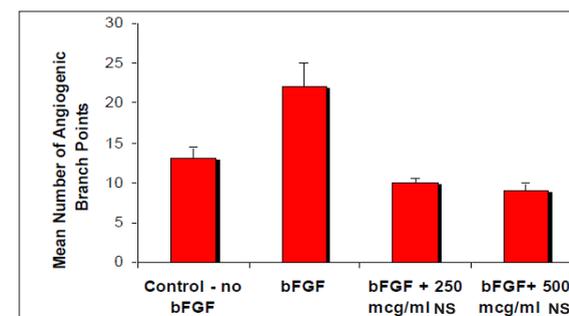


Figure 18

Expression of Pro-Angiogenic Factors: In addition, our in vitro studies demonstrated that NS decreased the expression of pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), angiopoietin-2, bFGF, platelet-derived growth factor (PDGF) and transforming growth factor (TGF β -1) in U2OS osteosarcoma cells.

Formation of capillaries: NS was also effective in another angiogenesis model demonstrating its inhibitory effect on the formation of blood vessels tubules by the human umbilical cord endothelial cells (HUVEC). In order to form a capillary the endothelial cells need to migrate in the tissue with the help of proteolytic enzymes and assembly in a form of microscopic tubules.

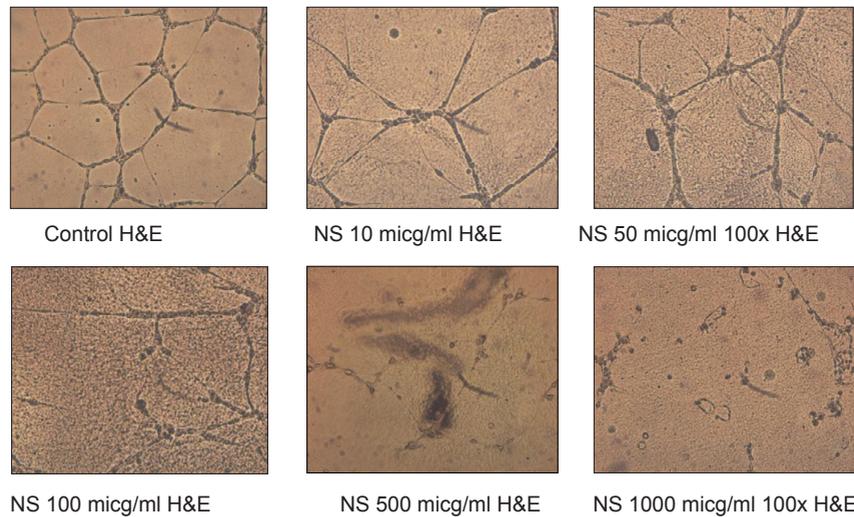
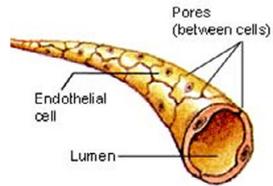


Figure 19

As shown in the figure 19 the endothelial cells in a standard environment can produce capillaries, seen under the microscope as a dense network of dark tubes. In contrast, when endothelial cells were exposed to different concentrations of NS a dose dependent inhibition of the capillary tube formation was observed. At the NS concentrations of 500 mcg/ml and 1000 mcg/ml no blood vessel structures were formed (Fig. 19).

Apoptosis

Apoptosis, also known as programmed cell death, is a complex process that occurs in several steps in every cell. This regular cycle of generation and death takes place in all normal cells, however, it is absent in cancer cells which makes cancer cells immortal. This lack of apoptosis in cancer cells makes them especially dangerous since each cell has the potential to divide endlessly.

Apoptosis is distinguished from necrosis by its characteristic morphological and biochemical changes. These changes include the compaction and fragmentation of nuclear chromatin, shrinkage of the cytoplasm and loss of membrane asymmetry. Several assays have been developed to study apoptosis one of them has been based on a distinctive feature of early stages of apoptosis, which is the activation of caspase enzymes. This family of specific proteases which plays a central role in apoptosis (e.g. caspases-3, -7, -8, -9, -10).

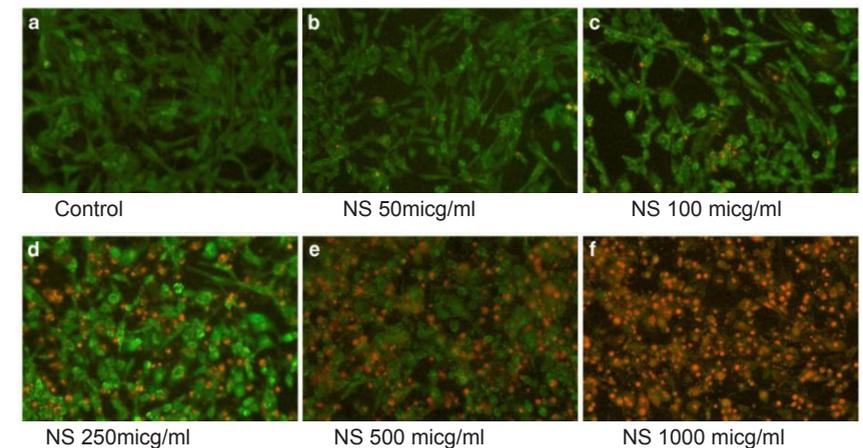


Figure 20

We studied the effects of NS on the induction of apoptosis in a number of cancer cell lines in vitro using a Live Green Caspases Detection method. The cancer cells were cultured in their recommended medium and treated with NS at different concentrations: 0, 100, 250, 500 and 1000 mcg/ml. All cells exposed to Live Green Poly Caspases were photographed under a fluorescence microscope and counted. Green-colored cells represented viable cells, yellow and orange cells early apoptosis, and red cells late apoptosis.

We observed a dose-dependent induction of apoptosis in a variety of cancer cells lines in the presence of NS. The example on Fig 19 show the effects of different concentration of NS on induction of apoptosis in rhabdomyosarcoma cells. At the highest NS concentration all cells appeared orange, indicating that the majority of these cancer cells were died. Quantitative analysis of live, early and late apoptotic cells is shown in Figure 21, which confirms that NS is effective in inducing natural death process in cancer cells.

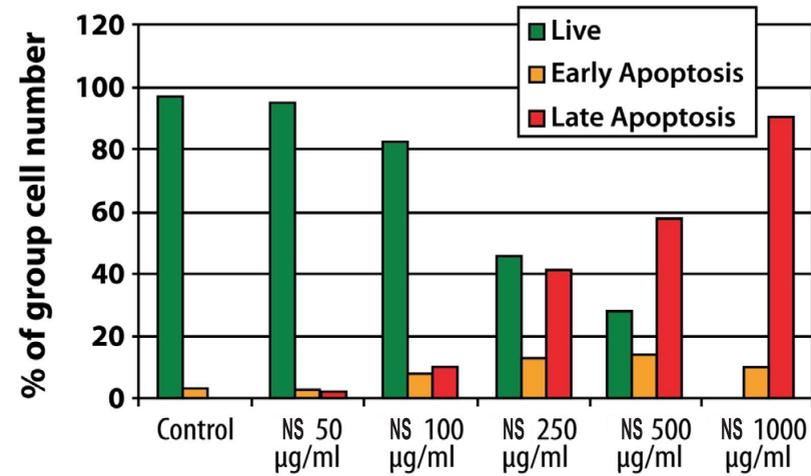


Figure 21



Conclusions

Our results suggest that NS is highly effective in targeting and inhibiting key mechanisms of cancer and it presents unique opportunity in fighting cancer worldwide.

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Dr. Rath Research Institute

The Dr. Rath Institute in Cellular Medicine is located in the Silicon Valley, in California. The Institute is staffed with experts handpicked from fields of medicine, biochemistry, and nutrition. Here, world-class scientists conduct innovative research utilizing the principle of nutrient synergy, and investigate the role of nutrients in preventing and treating a host of diseases.

Researchers at the Dr. Rath Research Institute are developing new scientific concepts based on Dr. Rath's discoveries in heart disease, cancer, infectious disease, and other diseases. Their scientific work has been published in various media around the world.

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This booklet is not intended as a substitute for the medical advice of a physician. The reader should regularly consult a physician in matters relating to his or her health and particularly in respect to any symptoms that may require diagnosis or medical attention.

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