

Induction of Apoptosis in the Human Prostate Cancer Cell Line DU-145 by a Novel Micronutrient Formulation



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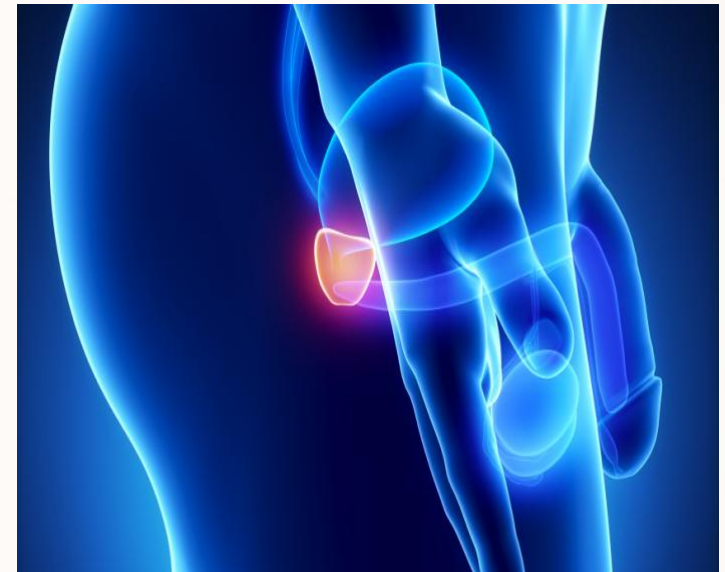
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Founder of the Dr. Rath Research Institute
Founder of the scientific concept of Cellular Medicine

PROSTATE

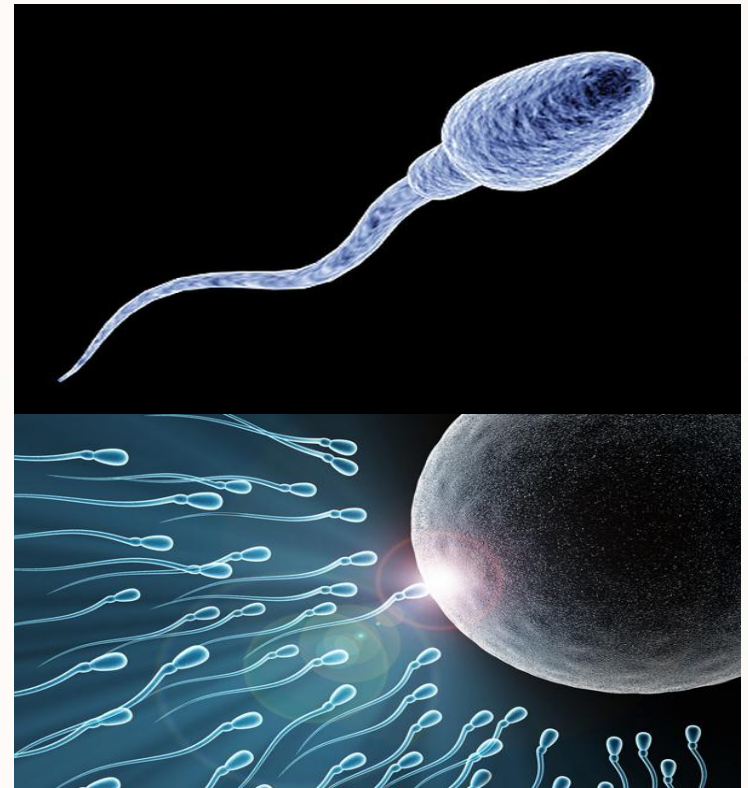
- Part of the male reproductive and urinary systems
- Located at the base of the bladder and in front of the rectum
- Partly muscular and partly glandular
- Size: varies from man to man, approximately 4 cm wide and 3 cm thick



FUNCTIONS

The thousands of glands building the prostate produce a fluid rich in minerals and proteins.

- The fluid protects and nourishes sperm cells (produced in the testicles)
- Serves as a transport medium for sperm in the vagina
- It helps to retain sperm in the vagina for an optimum period of time

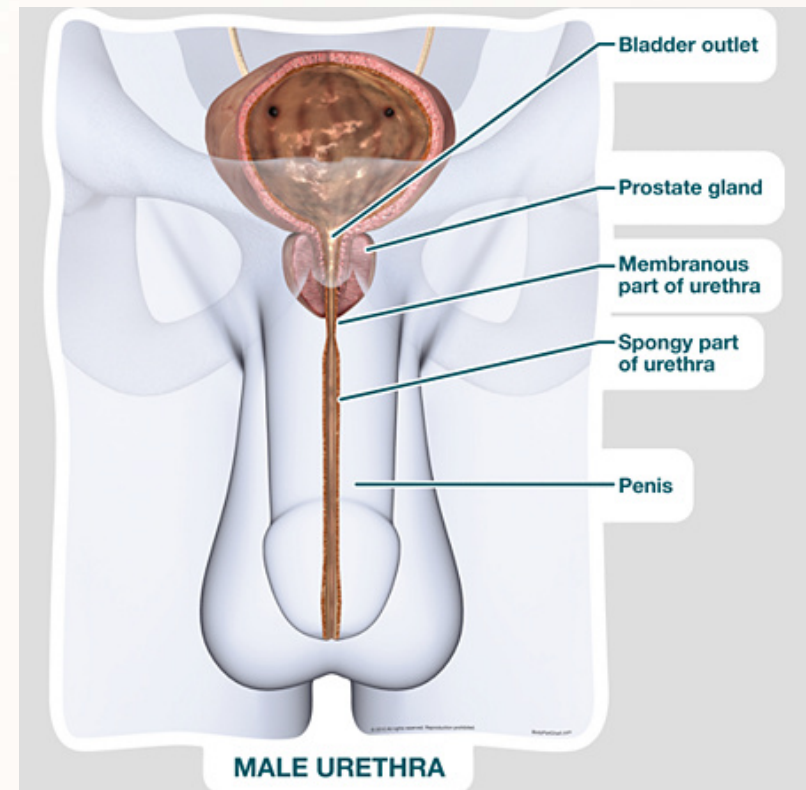


FUNCTIONS

The prostate fluid is secreted into the urethra during erection.

Urethra forms a tube from the bladder, through the prostate to the end of the penis. Urethra carries urine, semen and prostate fluids out of the body.

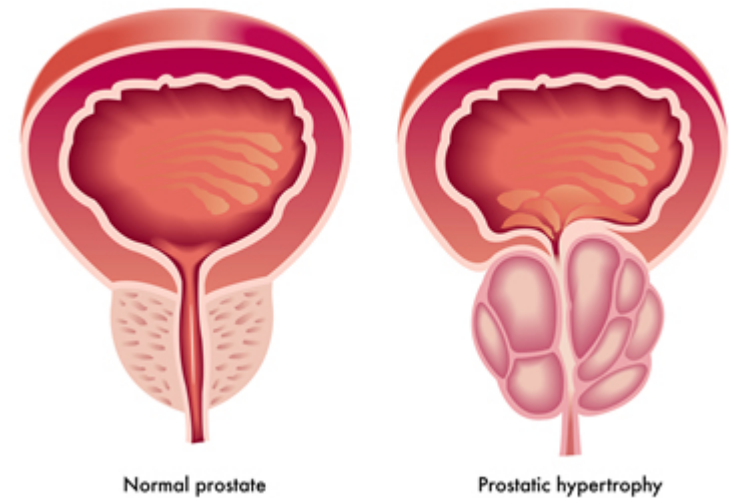
The prostate muscle fibers are involved in controlling a flow of urine.



GROWTH OF THE PROSTATE THROUGHOUT MAN'S LIFE

The growth of the prostate proceeds through two main stages:

- In puberty it doubles in size
- Around age 25 the second phase of growth begins and continues during most of man's life. During this stage the overgrowth of prostate - benign prostate hyperplasia (BPH) - can occur.



BENIGN PROSTATIC HYPERPLASIA (BPH)

BPH is a condition in which the prostate gland is enlarged and not cancerous. Usually it affects men older than age 50.

Benign Prostatic Hyperplasia

Symptoms

- Increased urination frequency and urgency
- Excessive urination at night
- Inability to pass urine, weakening of the urinary stream
- Sensation of poor bladder emptying
- Overflow incontinence
- Dribbling



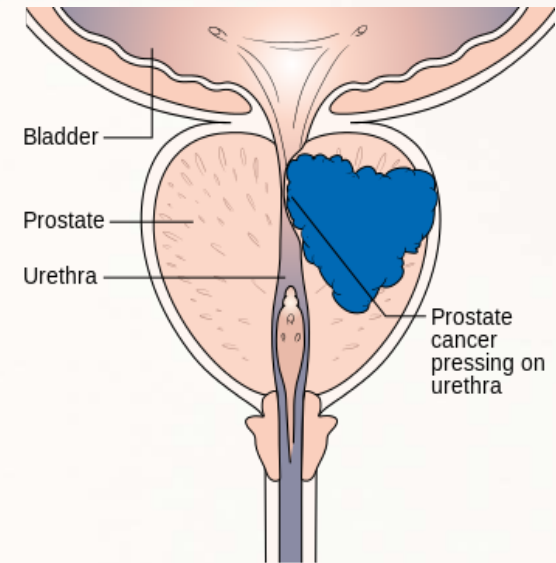
PROSTATE CANCER

Adenocarcinoma:

- A malignant tumor that in the majority of cases starts in the gland cells of the prostate (the cells producing the fluid added to the semen)
- Most prostate cancers grow slowly

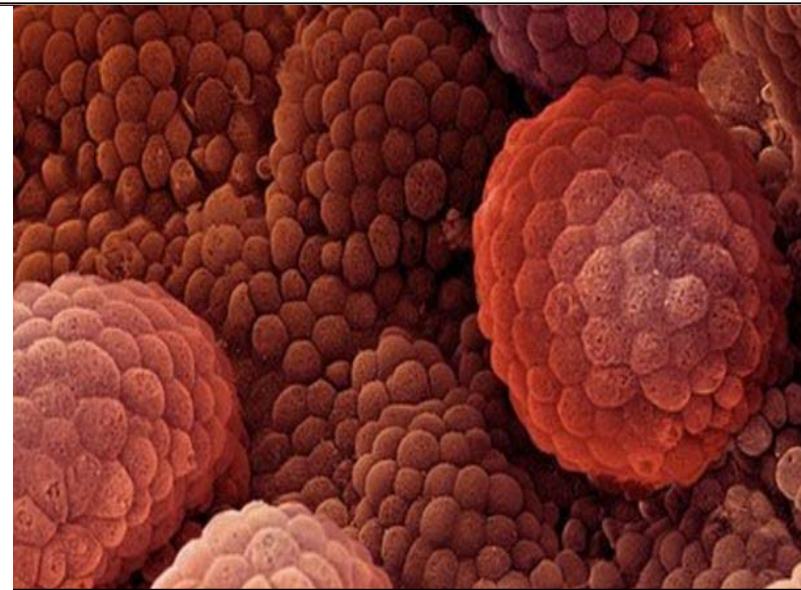
Pre-cancerous condition: Prostatic intraepithelial neoplasia (PIN)

- Prostate cells look abnormal but they do not spread to other parts of the prostate



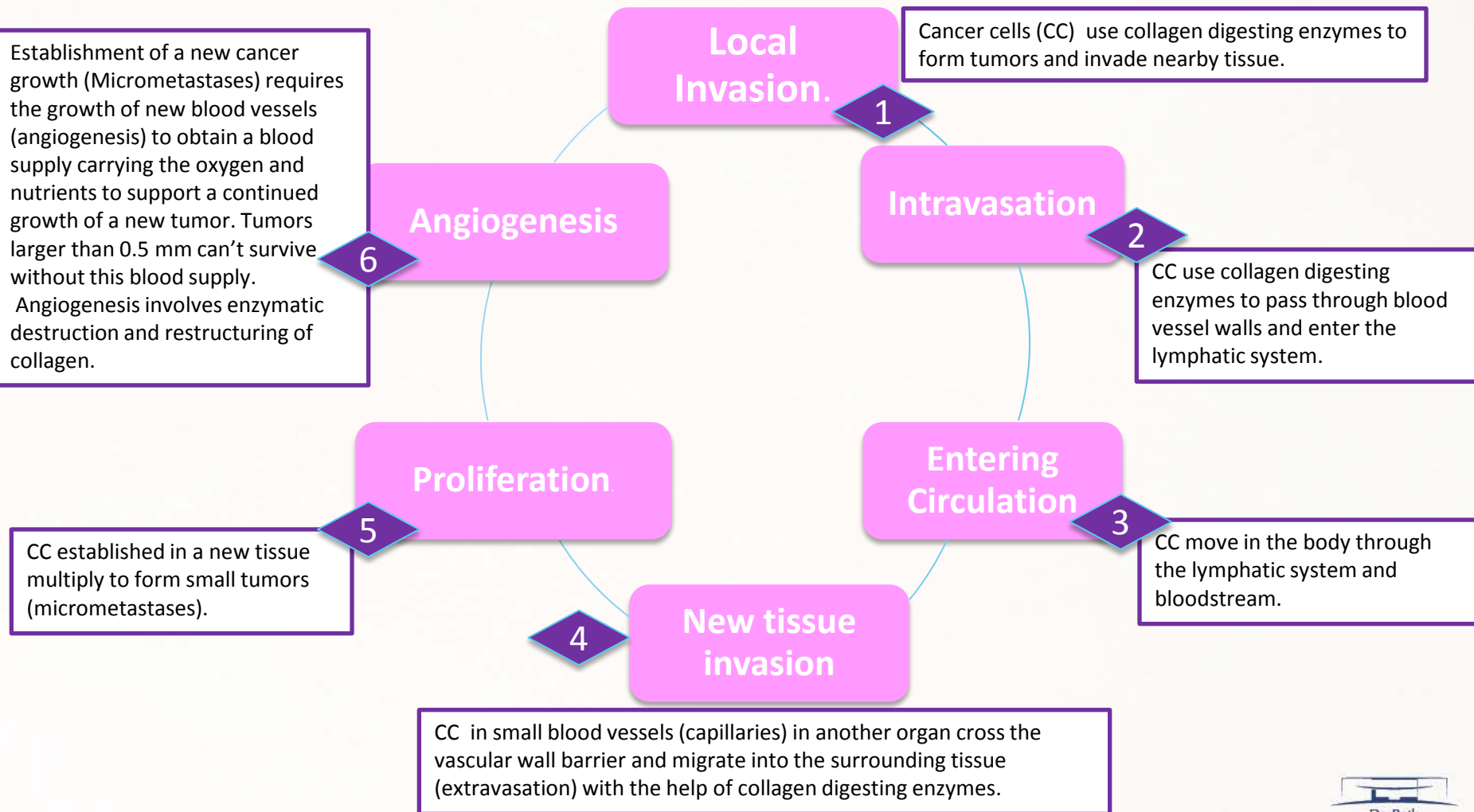
SYMPTOMS OF PROSTATE CANCER

- Problems passing urine
- Slow or weak urinary stream
- Frequent urination especially at night
- Painful urination
- Blood in urine
- Erectile dysfunction



Prostate cancer cells

Key Stages In Cancer Development



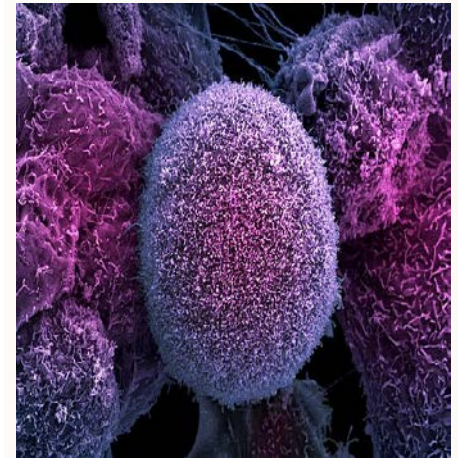
IMPORTANT CHARACTERISTIC OF CANCER CELLS

1. Continuous cell growth (proliferation)

Cancer cells lost an internal mechanism regulating their own growth and they multiply endlessly. This results in a continuous increase in the number of cells and the formation of tumors.

2. Immortality

Cancer cells lost an internal mechanism present in all cells which regulates their life-death cycle. The lack of this programmed cell death mechanism, called Apoptosis makes cancer cells immortal.



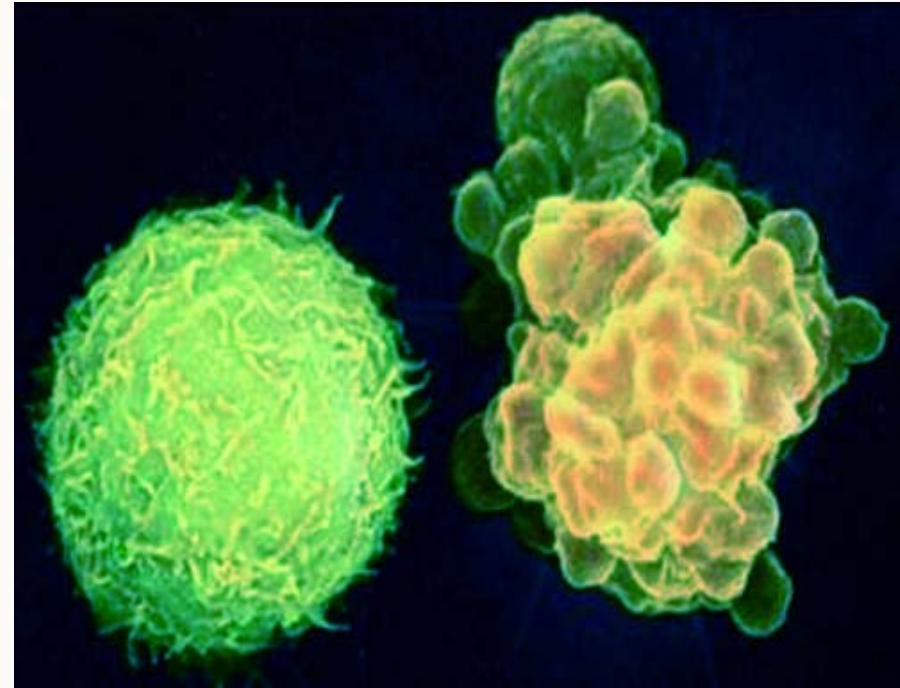
Targeting these cancer cells features might have therapeutic benefits

APOPTOSIS (PROGRAMMED CELL DEATH)

- Apoptosis encoded in every cell determines their life span.
- It evolved as a rapid and irreversible process to effectively eliminate cells that become dysfunctional or abnormal
- It involves self-activating death program in which cells commit suicide in several distinct steps.

Cancer cells

- Evade apoptosis
- Immortality facilitates tumor development and resistance to many therapies




The picture above shows a healthy cell (left) and a cell in a late stage of the apoptotic process with undergoing fragmentation and gradual structural demolition.

CONVENTIONAL TREATMENTS

- **Surgery:** successful for treating early stage cancer, however is limited to localized tumors and does not assure a removal of all cancer cells, which can potentially grow forming new tumors . Risk of nerve damage (impotence, incontinence) or rectal injury
- **Hormonal therapy:** blocks the action of cancer supporting male hormones (androgens). Only effective till the cancer can spread independent of male hormones
- **Radiotherapy:** uses ionizing radiation to kill cells. It is ineffective in later stages of cancer. By damaging also healthy cells it increases risk of new cancers and weakens the immune system. Associated with many side effects.

There is an urgent need for safe and effective new therapeutic approaches

Diet and Prostate Cancer

A photograph of various fresh vegetables arranged in baskets and bowls. The vegetables include green leafy greens, dark purple eggplants, yellow beans, green beans, red tomatoes, cucumbers, and a head of cauliflower. The background is a dark, textured surface.

Consumption of a plant based diet has been associated with the prevention of prostate cancer. Diet and nutritional supplementation have been investigated for their beneficial effects in prevention and attenuation of prostate cancer.

Cellular Medicine Approach

Cellular medicine strategy in natural control of cancer development and its spread has been based on selection of critical biological targets involved in cancer and application of micronutrient synergy for their effective control.

We developed a unique combination of naturally occurring micronutrients (NM) containing vitamins, amino acids, plant extracts, and trace elements and demonstrated its efficacy against key mechanisms of cancer. Its advantages include:

- **Universality:** anti-cancer efficacy in over 50 types of human cancer cells.
- **Pleiotropic effects:** Affecting several cancer mechanisms at once
- **Safety**



Current study

Our earlier study found that dietary supplementation with NM significantly inhibited the development of prostate tumors in nude mice.

In the current study we investigated whether the inhibition of prostate tumors by NM includes an anti-proliferative and pro-apoptotic mechanisms triggered by NM.



For earlier studies visit:
www.drrathresearch.org

Composition of the Nutrient Mixture (NM) Tested in the Study

The nutrient mixture (NM) contains the following micronutrients in the relative amounts as indicated below:

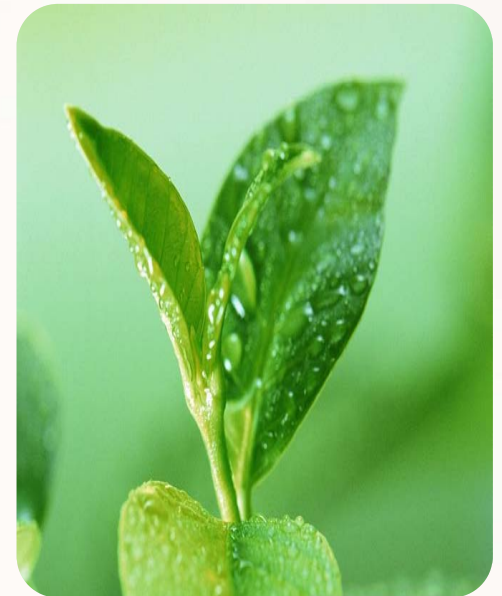
- Vitamin C, 700 mg
(as ascorbic acid, Magnesium and Calcium ascorbates and scorbyl palmitate)
- L-lysine, 1000 mg
- L-proline, 750 mg
- L-arginine, 500 mg
- N-acetyl cysteine, 200 mg
- Standardized green tea extract (80% polyphenols), 1000 mg
- Selenium, 30 μ g
- Copper, 2 mg
- Manganese, 1 mg

Methods Used in the Study (In Vitro)

Cancer cells of a specific human prostate cell type were grown in tissue culture media.

Cells were either kept under standard experimental conditions (control) or exposed to different concentrations of the nutrient mixture in the cell medium (test):

- **Control** group: cells left untreated
- **Test** groups: cells treated with the nutrient mixture at 100, 250, 500, and 1000 $\mu\text{g}/\text{ml}$ concentrations for 24 hours





The study evaluated the following aspects of NM in prostate cancer cells:

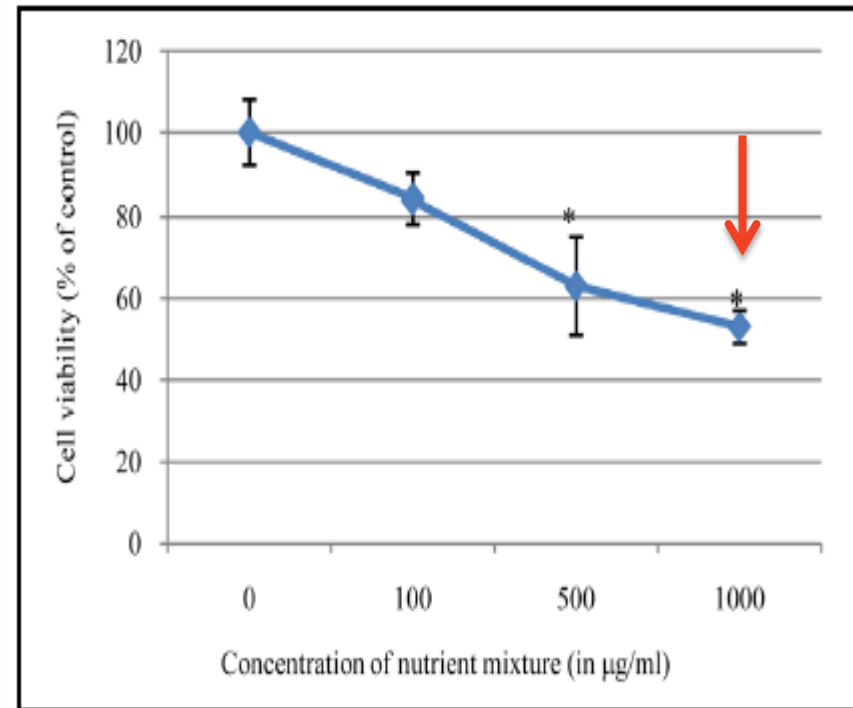
- Viability of prostate cancer cells exposed to NM
- Induction of apoptosis in prostate cancer cells and normal cells by the NM
- Nutrient mixture induced morphological changes associated with apoptosis in these cells

NM DECREASED THE VIABILITY OF PROSTATE CANCER CELLS

Exposure of prostate cancer cell to increasing concentrations of NM for 24 hours reduced their survival:

Accordingly:

- at 100 $\mu\text{g/ml}$ NM about 84% cells survived
- at 500 $\mu\text{g/ml}$ NM about 63% cells survived
- at 1000 $\mu\text{g/ml}$ NM only about half (53%) cells survived



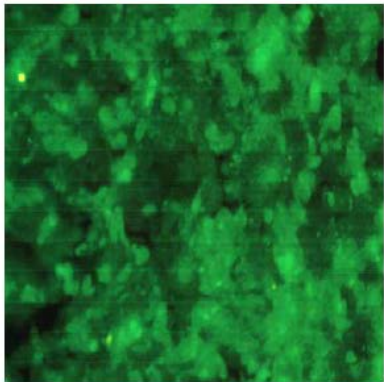
NM INDUCED APOPTOSIS IN PROSTATE CANCER CELLS

Different stages of cell apoptosis can be visualized using a specific detection system which allows to evaluate the advancement of cell death process by observing the changes in fluorescence of a specific compound added to the cells. In this test all alive (viable) cells are colored green. The color of apoptotic cells transitions from yellow to orange, red and dark red according to the advancement of their death.

This method allows for both qualitative and quantitative assessment of apoptosis by observing the cells under the microscope.

The following slides illustrate that the exposure of prostate cancer cells to increasing NM concentrations result in a corresponding decrease in percentage of alive cells and an increase in percentage of cells undergoing late apoptotic stages (dying).

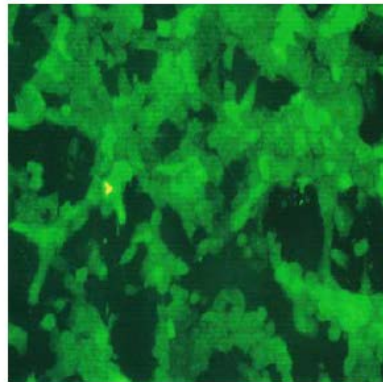
NM INDUCED APOPTOSIS IN PROSTATE CANCER CELLS (Qualitative results)



**Control
(without NM)**

No apoptosis

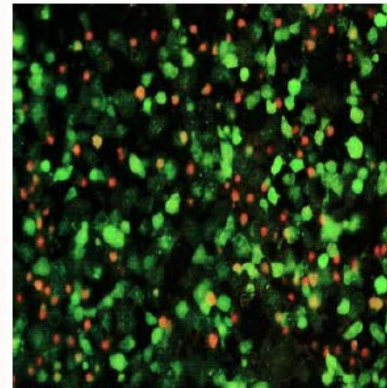
All cells are colored green, which means that they are viable



**Treated with
100 µg/ml NM**

Slight apoptosis.

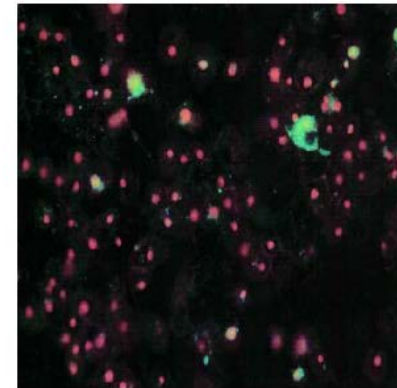
Most cells are viable (green) and about 6% cells are in early phase of apoptosis (yellow)



**Treated with
500 µg/ml NM**

Moderate apoptosis.

The mixture of green, yellow and orange cells. About 49% cells undergo apoptosis (about 39% are in late phase)



**Treated with
1000 µg/ml NM**

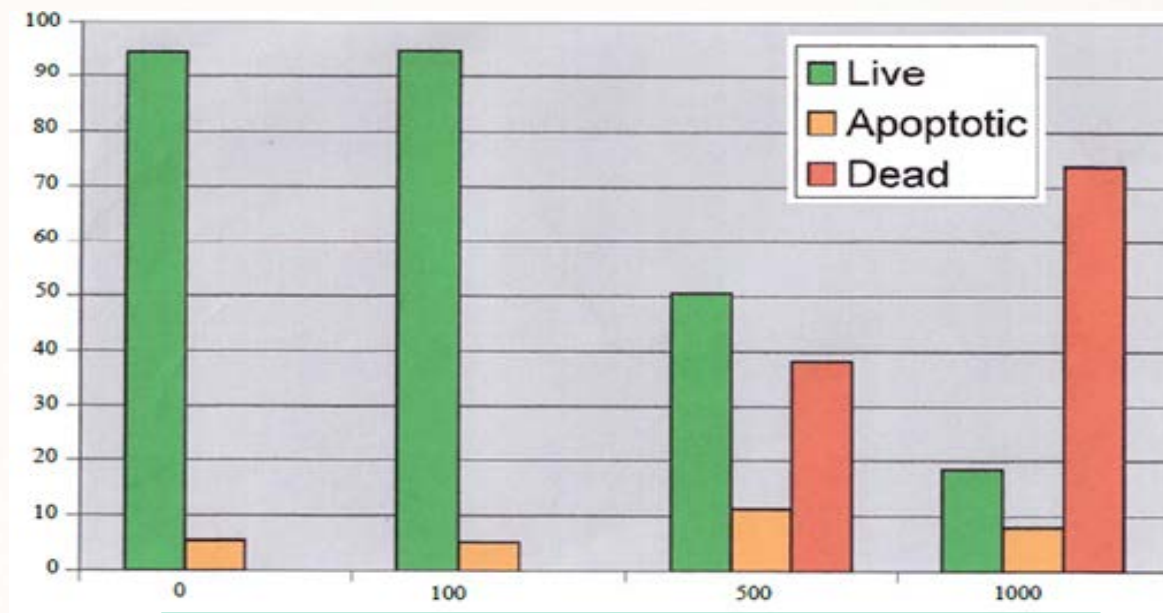
Advanced apoptosis.

Most cells visible in red color. About 83% are at apoptosis (about 75% are in late phase)

NM INDUCED APOPTOSIS IN PROSTATE CANCER CELLS (Quantitative results)

Distribution of prostate cancer cells in different phases of apoptosis

% of cells in different apoptotic phases



Concentrations of nutrient mixture in (µg/ml)

Detailed calculations of % of alive, early and late apoptotic prostate cancer cells based on the results shown on a previous slide

NM INDUCED MORPHOLOGICAL CHANGES ASSOCIATED WITH APOPTOSIS

Prostate cancer cells exposed to increasing concentrations of NM showed characteristic morphological changes associated with apoptosis:

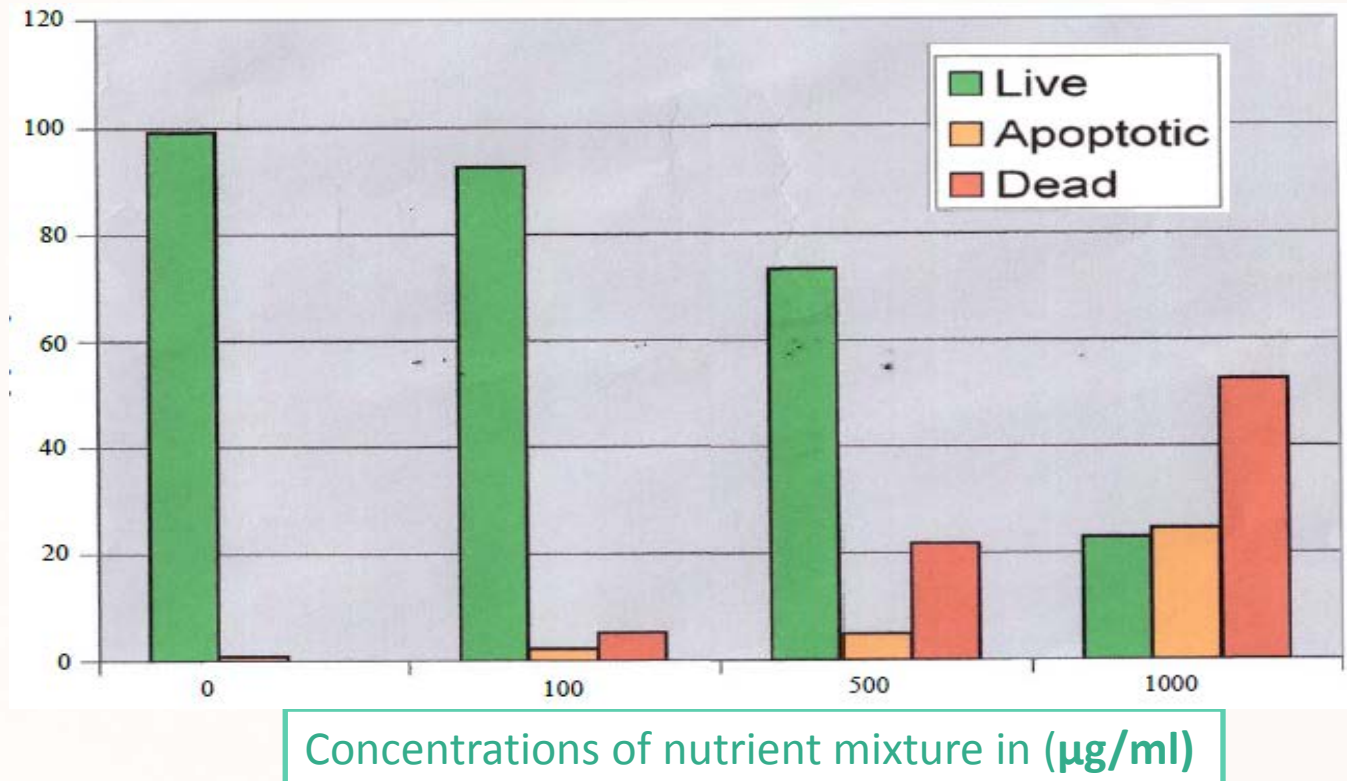
- Extensive cell shrinkage, rounding and nuclear condensation
- These changes were dose-dependent showing an increase in intensity with the exposure to higher NM concentrations and corroborated with the degree of apoptosis.

HOW NM AFFECTS APOPTOSIS IN NORMAL HEALTHY CELLS

In the study we also compared the effects of various NM concentrations on the induction of apoptosis in healthy cells (normal human dermal fibroblasts).

There was a corresponding decrease in percentage of live cells and an increase in percentage of cells undergoing various apoptotic stages with increasing NM concentrations. However, normal cells were much less susceptible to apoptosis than prostate cancer cells at all corresponding NM concentrations.

NM INDUCED APOPTOSIS IN HEALTHY CELLS



% of cells in apoptotic phases

Concentrations of nutrient mixture in (µg/ml)

The results show that up to 500 µg/ml NM a small percentage of normal fibroblasts cells undergo apoptosis. At NM 1000 µg/ml much less normal cells compared to prostate cancer cells is at apoptosis (compare slide #23)

SUMMARY



1. Exposure of prostate cancer cells to the specific mixture of micronutrients caused a dose dependent:
 - Decrease in cell viability
 - Induction of apoptosis and morphological changes characteristics of apoptotic cells
2. The NM induced apoptosis also in normal fibroblasts but at much lower level than in prostate cancer cells. Since normal cells located in proximity of cancer cells can aid some cancer promoting mechanisms (i.e., degradation of connective tissue) this may be of additional value in cancer control. However, the effective concentrations of NM should be at the 500 $\mu\text{g}/\text{ml}$.
3. This study should be viewed in the context of our previous in vivo findings which showed the NM efficacy in decreasing growth of prostate cancer cells and formation of tumors as well as in curbing various pro-angiogenic and metastatic factors without adverse health effects.

IMPLICATIONS OF THESE FINDINGS



The nutrient mixture tested in this study confirmed its potent anticancer activity by demonstrating its ability in inhibiting prostate cancer cells viability and inducing apoptosis.

The results imply that dietary intake of the NM should be considered in developing safe and effective approaches to natural control of prostate cancer.