

Effect of a nutrient mixture on the localization of extracellular matrix proteins in HeLa human cervical cancer xenografts in female nude mice



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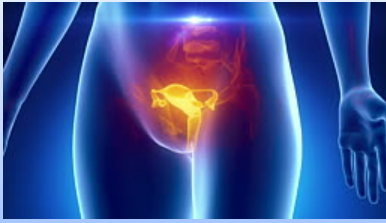
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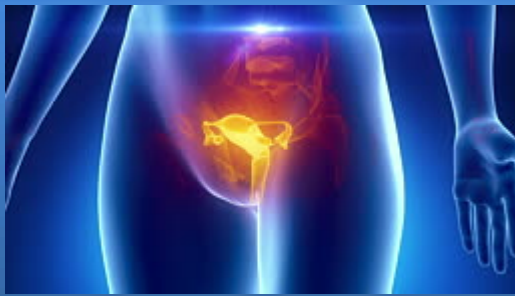
Founder of Dr. Rath Research Institute
Founder of the scientific concept of Cellular Medicine



Cervical Cancer

- ❑ Cancer in the cells of the cervix- the lower part of the uterus that connects to the vagina.
- ❑ Cells in the cervix grow out of control and invade nearby tissues or spread throughout the body.
- ❑ It has various causes but is frequently linked to the human papillomavirus (HPV) infection, which is the common virus that spreads through sexual intercourse.
- ❑ There are over 100 types of HPV, but only a few strains have been associated with the presence of cancerous cells.

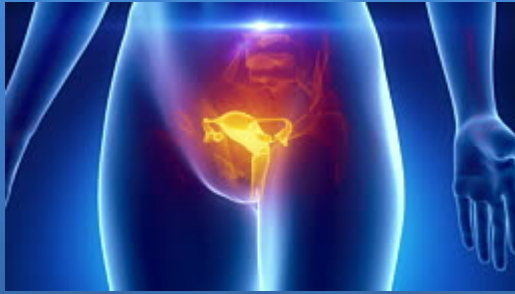




Human Papilloma Virus (HPV)

- ❑ In many cases, an HPV infection will heal on its own.
- ❑ However, if HPV persists, it can lead to development of pre-cancerous cells, which if left not detected and treated, they can progress to cervical cancer

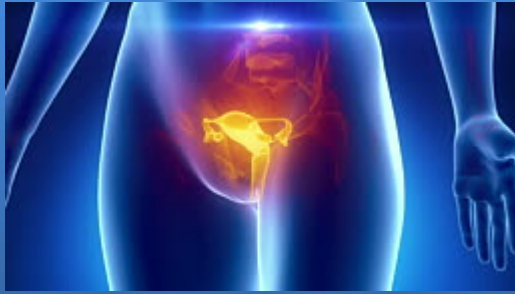




Impact of Cervical Cancer

- The third most commonly diagnosed cancer and the fourth most common cause of mortality in women worldwide.
- Most cases of cervical cancer occur in women older than 35, it rarely occurs in women younger than 20.



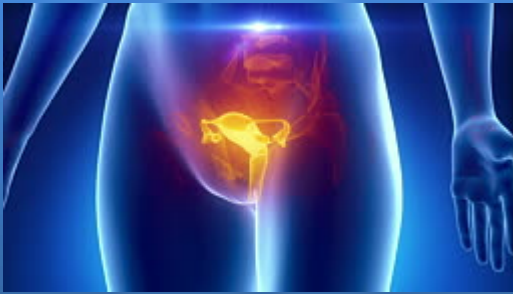


Symptoms of Cervical Cancer

Once cervical cancer progresses into more advanced stages, symptoms begin to appear, including:

- Pelvic pain, unrelated to other conditions, menstruation, or physical exertion
- Continuous vaginal discharge, (pale, watery, pink, brown, or foul-smelling)
- Pain during sexual intercourse
- Abnormal vaginal bleeding





Physiology of the Cervix

The Cervix is built of:

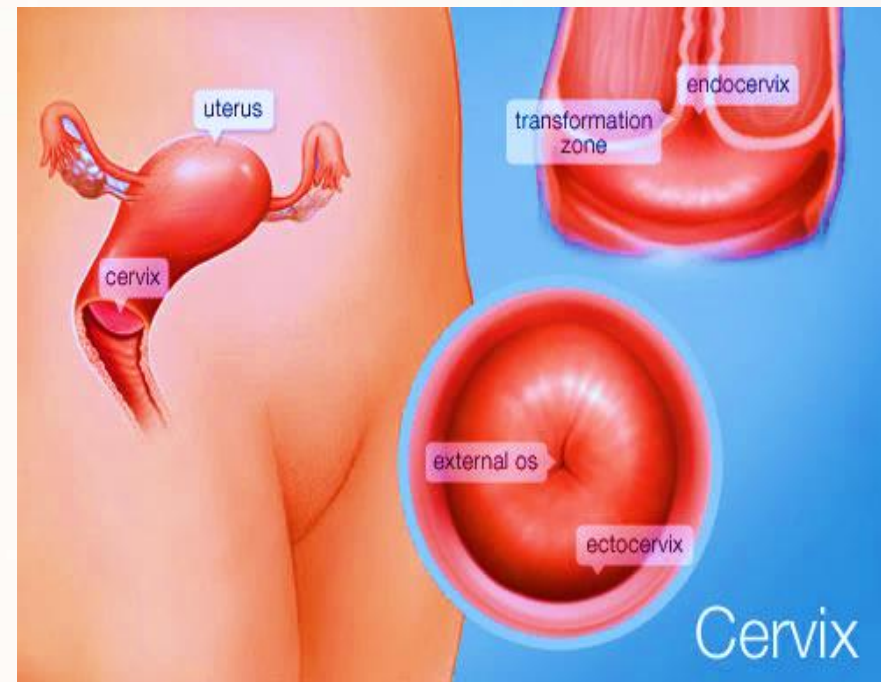
- The Ectocervix:

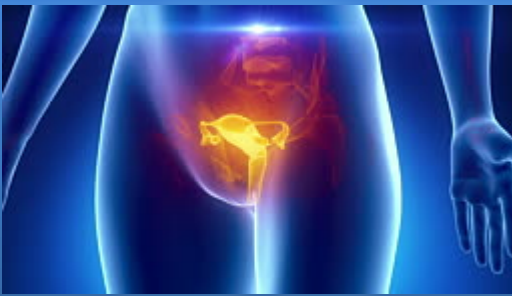
The part of the cervix visible through the vaginal canal.

- The Endocervix/ endocervical canal:

The internal, tunnel-like part of the cervix, which opens into the uterus.

The overlapping border between the endocervix and ectocervix is also known as **the transformation zone**.

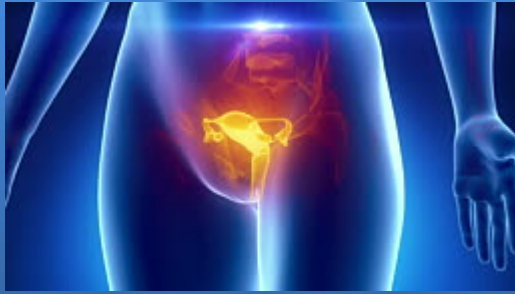




Cervix - function

- 1. Menstruation:** during menstruation, the cervix opens slightly, allowing the passage of menstrual blood from the uterus into the vagina. The stretching causes cramps and some women experience less pain after giving birth due to the widening of the cervical opening.
- 2. Sperm storage:** promotes fertility. Directs the sperms into the uterus during intercourse.
- 3. Protective barrier:** protects the uterus, the upper reproductive tract and a developing fetus from pathogens.





Cervical Cancer Therapy

Early detection of cancerous cells has a significant impact on morbidity and mortality rates.

Cervical cancer usually develops very slowly. It typically takes 10-15 years for the pre-cancerous condition called dysplasia to develop into cancer.

Cervical cancer can be treatable in early stages (average 5 year survival rate in early detection is 92%) but once it has metastasized, the outcome is very poor.

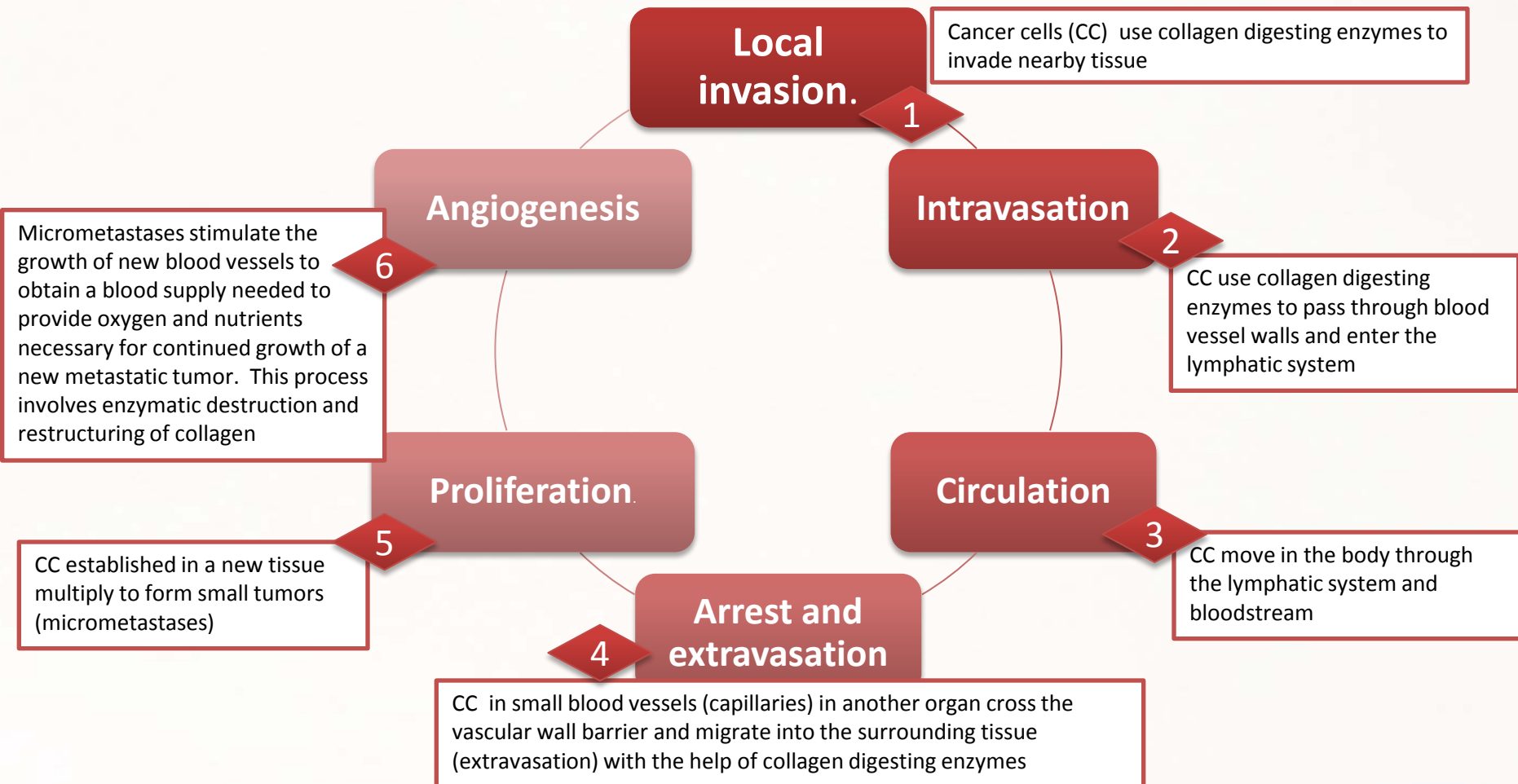


Conventional treatments involving surgery, radiation and chemotherapy are associated with severe and often debilitating side effects and can trigger new cancers.

There is a need for effective, safe and affordable approaches to cervical cancer.

TUMOR INVASION AND METASTASIS

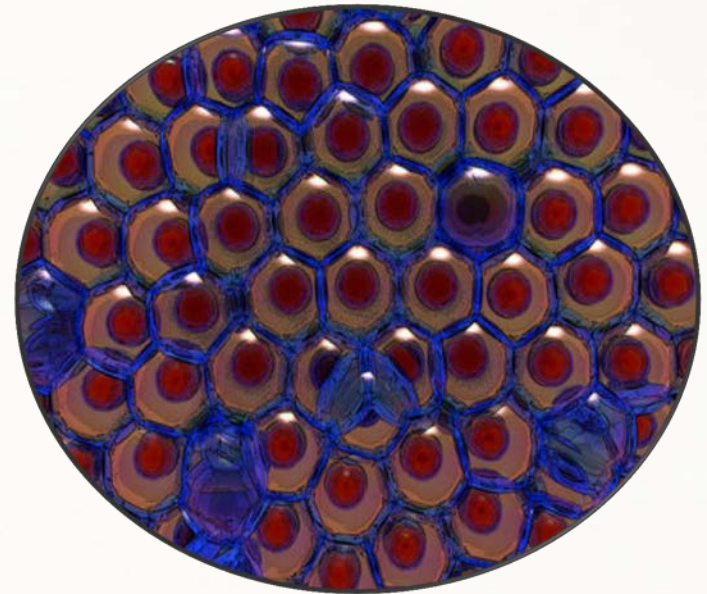
Key steps in cancer invasion and metastasis



TUMOR INVASION AND METASTASIS

BREAKING EXTRACELLULAR MATRIX INTEGRITY

- Cells of our body are embedded in a network of collagen and other connective tissue molecules called extracellular matrix (ECM) which keep them in place.
- The ECM comprises collagen, proteoglycans, fibronectin, laminin and other glycoproteins.
- In order to migrate through the network, a cell has to break down this dense network of connective tissue.
- Cells secrete enzymes that break down the extracellular matrix dissolving the surrounding tissue (collagen and elastin fibers).



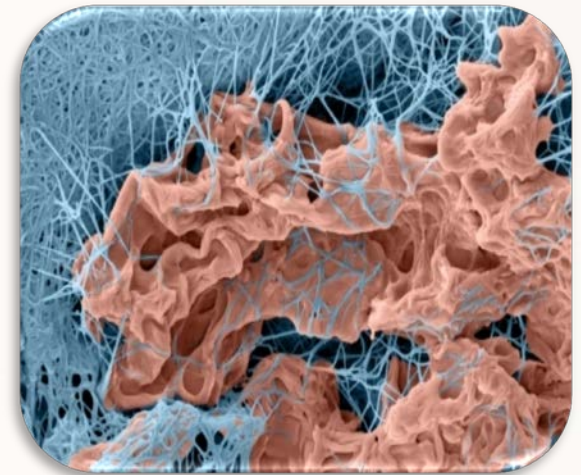
TUMOR INVASION AND METASTASIS

CONNECTIVE TISSUE DISSOLVING ENZYMES

There are several types of enzymes that cancer cells use to digest connective tissue including:

- ❑ Urokinase also called Urokinase-type Plasminogen Activator (uPA)
- ❑ Matrix Metalloproteinases (MMPs)

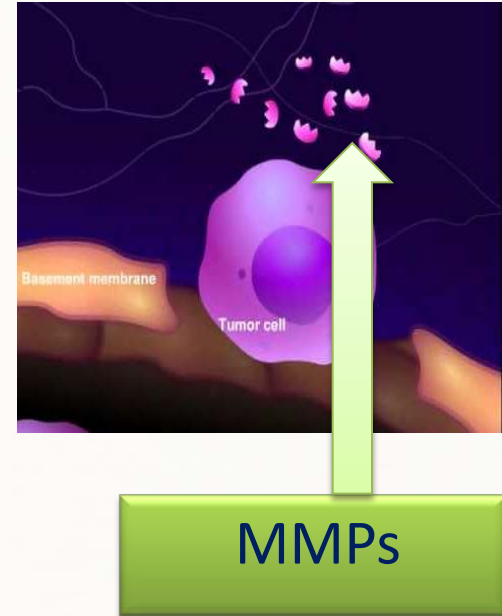
The more collagen digesting enzymes a cancer cell produces, the more aggressive the cancer is and the faster it spreads through the body.



TUMOR INVASION AND METASTASIS

– MATRIX METALLOPROTEINASES (MMPs)

- MMPs represent a family of enzymes responsible for the breakdown of connective tissue proteins.
- Excessive MMPs expression, especially MMP-2 and MMP-9, play a key role in promoting tumor cell invasion and metastasis.
- MMP-2 and MMP-9 degrade type IV collagen, a major component of the basement membranes.
- Invasive tumors are characterized by the presence of thinned, fragmented and disrupted basement membranes - specially their collagen IV and laminin components.



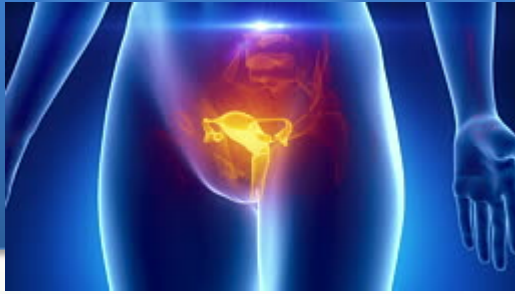
Preventing uncontrolled connective tissue degradation by nutrients forms the basis of the universal, safe and effective approach to prevent growth and expansion of tumors

We have developed a unique synergistically acting combination of micronutrients (NM) that was shown to be effective in controlling key mechanisms of cancer including its growth and spread in the body. This nutrient mixture comprises lysine, proline, ascorbic acid, green tea extract and other natural components.

- Our earlier studies (*In Vitro*) showed that NM could significantly inhibit HeLa cervical cancer cell proliferation.
- The present study (*In Vivo*) investigates the effect of NM dietary supplementation on growth of cervical tumors and accompanying it changes in various ECM proteins in female nude mice.



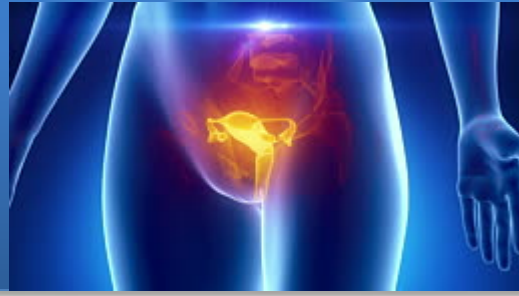
Study objective



Investigate the effect of dietary supplementation with a nutrient mixture on the development of cervical cancer by implanting HeLa cancer cells in nude female mice.



Nutrient mixture (NM): lysine, ascorbic acid, proline, green tea extract and other micronutrients. The NM simultaneously acts on critical physiological targets in cancer progression and metastasis.

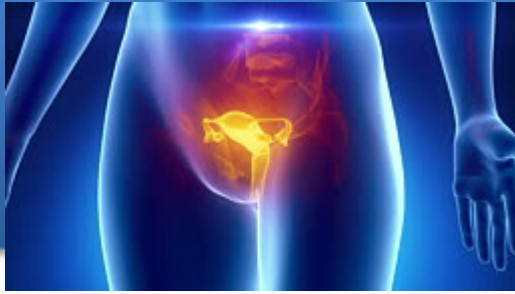


Parameters evaluated in the study

Morphological changes in ECM protein composition of tumors in relation to NM supplementation. The evaluation included collagen I, collagen IV, fibronectin, laminin, periodic acid-Schiff (PAS) and elastin

- **Collagen:** the main structural protein and main component of connective tissue (I) and basement membranes (IV).
- **Fibronectin:**
 - A high molecular weight glycoprotein, binds ECM components such as collagen and fibrin.
 - Affects cellular interactions with the ECM. Important in cell adhesion, migration, growth and differentiation.
 - Altered fibronectin expression, degradation and organization have been associated with cancer progression.
- **Laminin:** high molecular weight proteins of the ECM, affects cell differentiation, migration and adhesion as well as phenotype and survival.
- **Elastin:** a highly elastic protein, allows the tissues in the body to resume their shape after stretching or contracting.

Method: In Vivo Study



12 female nude mice – 5 to 6 weeks old– were inoculated subcutaneously with HeLa cells.

Following the inoculation, the mice were randomly divided into two groups:

1. **Control group:** mice were fed regular mouse chow.
2. **NM group:** mice regular diet was supplemented with 0.5 % NM. Since mice consumed about 4 g of food/day during the study, thus, the supplemented mice received ~20 mg NM per day.

After 4 weeks, the mice were anesthetized/sacrificed, the tumors were excised, weighed and processed for histological examination.



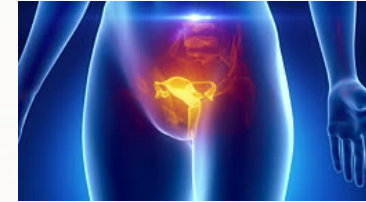
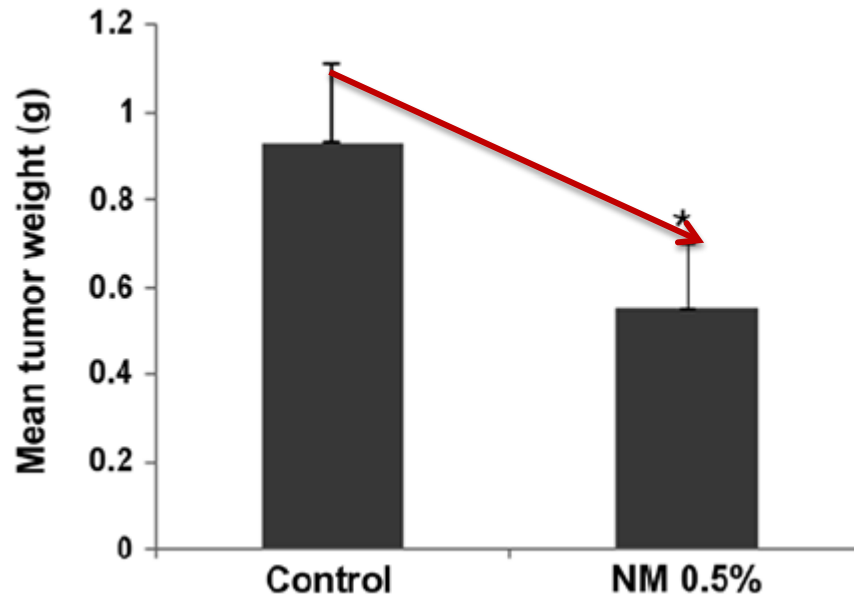


Effect of NM dietary supplementation on:

1. Tumor growth
2. Collagen I and IV deposition in tumors
3. Fibronectin expression in tumors
4. Laminin expression in tumors
5. PAS in tumors

Results in vivo study

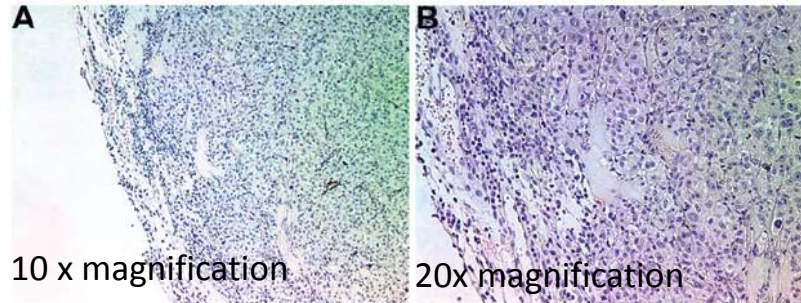
NM Supplementation Inhibited Growth of Cervical Tumors



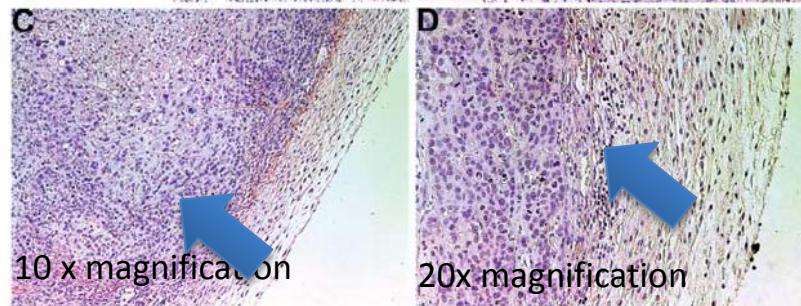
Mice fed a diet supplemented with 0.5% NM, developed smaller tumors compared to control mice. Tumor weight was inhibited by 59 % ($p = .001$), which means that this change was statistically significant.

Effect of NM supplementation on Collagen I

Control



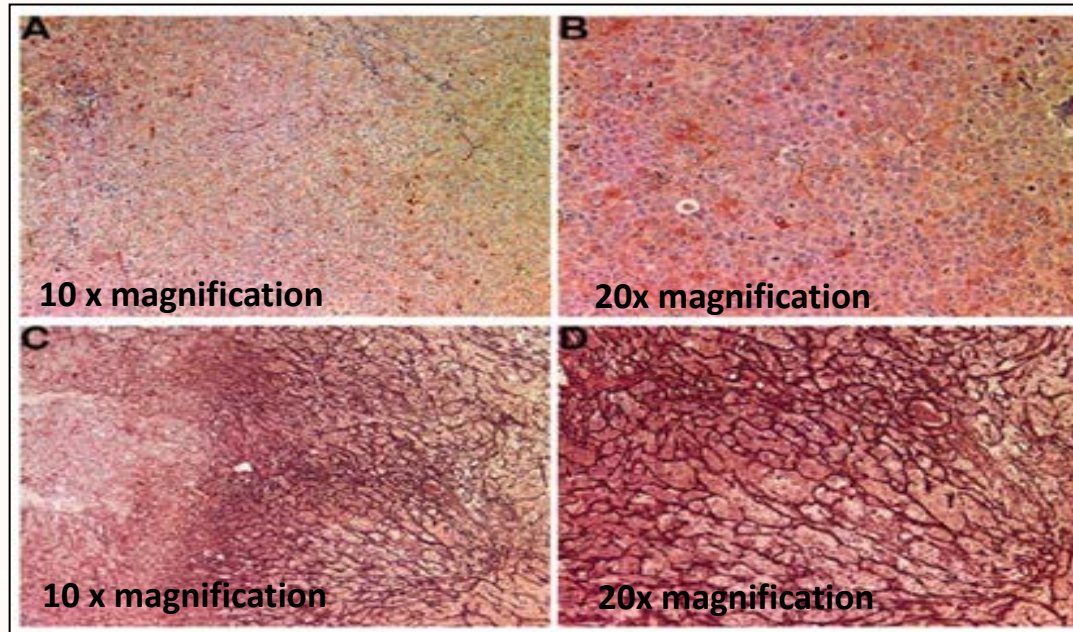
NM
supplemented



- Tumors from control mice had low collagen I expression (purple color) both inside a tumor mass and its surrounding did not show a well formed capsule (A & B).
- Tumors from the NM-supplemented mice had more collagen I within the tumor. In addition they formed a thick fibrous capsule surrounding the tumor (C & D), which forms a barrier curtailing spread of cancer cells.

Effect of NM supplementation on Collagen IV (collagen in base membranes)

Control

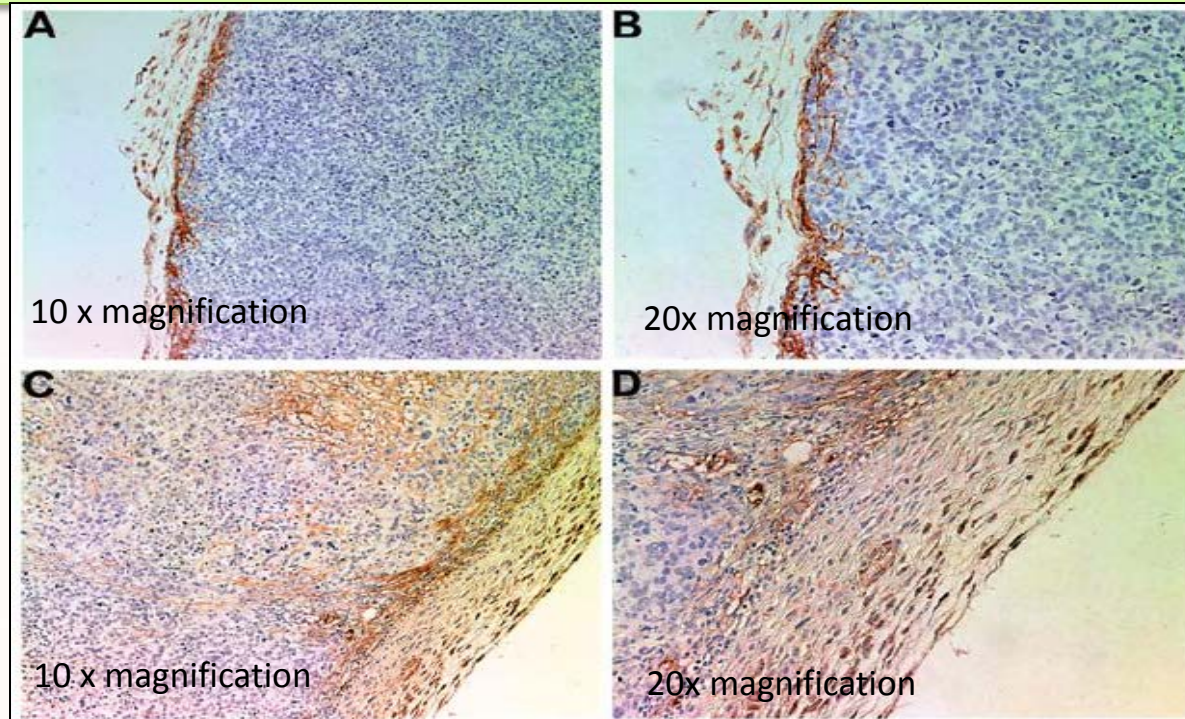


NM
supplemented

- Tumors developed in Control mice showed a diffused cytoplasmic and capsular collagen IV distribution with abundant nucleated cells (A & B).
- NM supplementation resulted in a substantial increase in collagen IV production and a formation of a dense fibrous network of collagen IV creating chambers that surrounded live nucleated cells and large amounts of necrotic cell debris (C & D). The fibrotic network of collagen IV became denser towards the core of the tumor and its increased density was associated with greater amount of cell necrosis.

Effect of NM supplementation on Fibronectin

Control



10 x magnification

20x magnification

10 x magnification

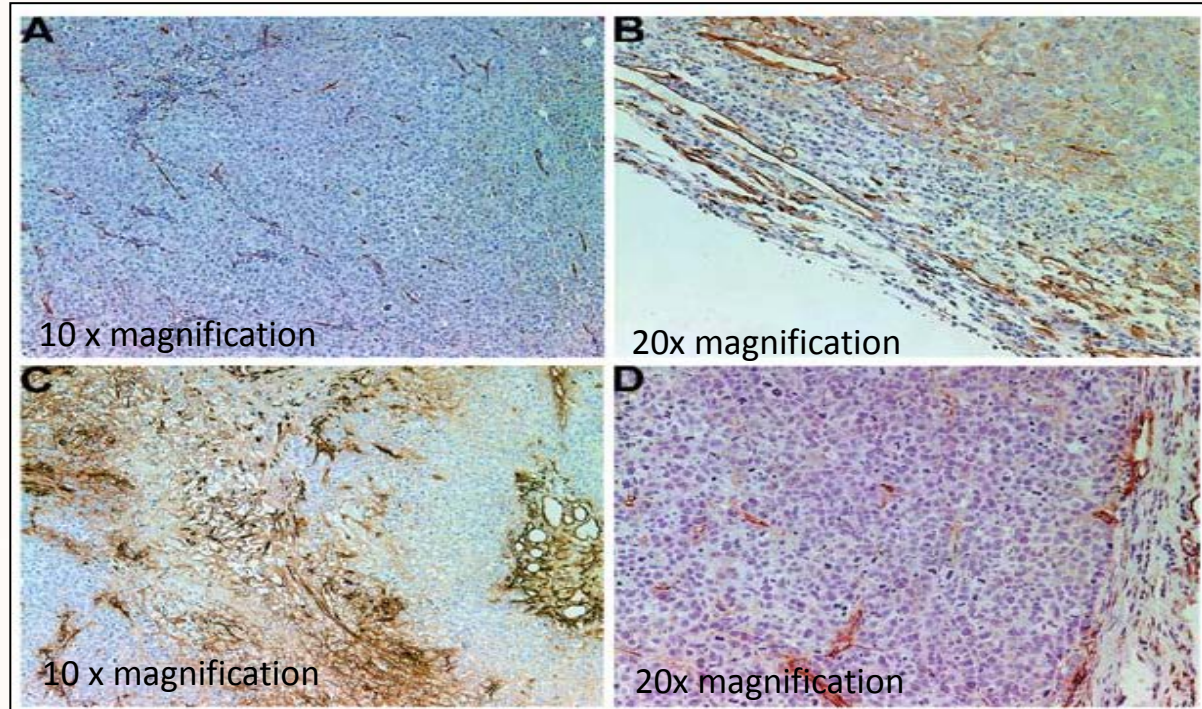
20x magnification

NM
supplemented

- In tumors developed in control mice there was less fibronectin (brown spots) in the tumor mass and only a thin fibrous fibronectin capsule was present.
- Tumors from the NM-supplemented mice showed a well-defined border containing fibronectin in the external capsule and intense areas of staining inside a tumor mass.

Effect of NM supplementation on Laminin

Control

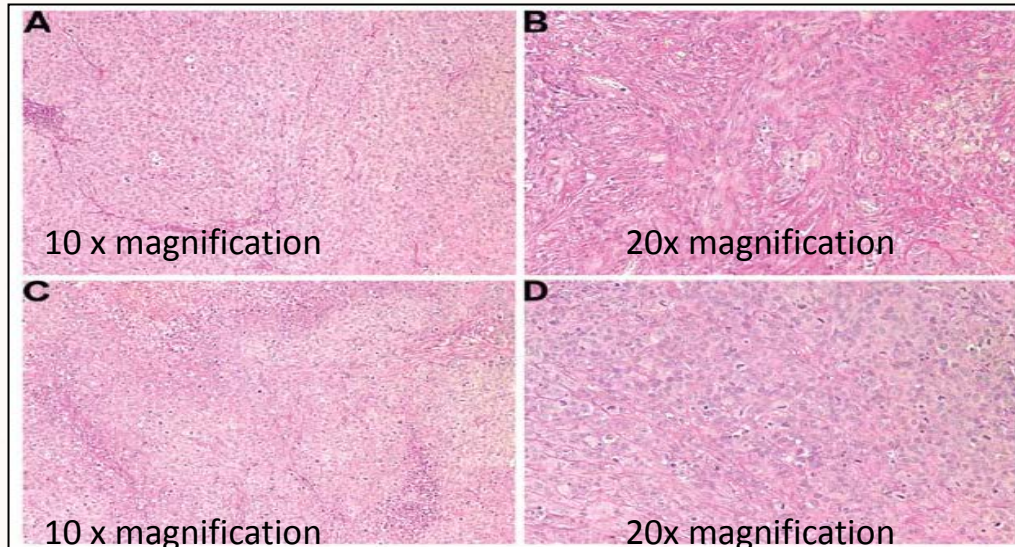


NM
supplemented

Laminin appeared abundantly distributed in both control and NM-treated tumors, however, tumors from NM-supplemented group displayed a chamber-like network of laminin within a tumor mass.

Effect of NM supplementation on PAS and Elastin

Control



NM
supplemented

Tumors from control group showed f intense PAS staining (presence of polysaccharides and glycoproteins) within a tumor mass, whereas tumors from the NM treated mice exhibited a more uniform diffuse pattern of PAS expression. This helps in better classification of tumor types.

No differences in Elastin was detected in tumors from control and NM-supplemented groups.

CONCLUSION



- ✓ This micronutrient mixture was effective in significantly inhibiting tumor growth accompanied by characteristic changes in specific extracellular matrix proteins distribution towards confining growth of tumors.
- ✓ This nutrient mixture does not show any toxic effects.
- ✓ Dietary intake of the mixture of micronutrients should be considered in developing safe and effective approaches to control Cervical cancers.